Sorption and degradation of pesticides in biopurification systems

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UNIVERSITEIT GENT

FACULTEIT **BIO-INGENIEURSWETENSCHAPPEN**

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Sorptie en afbraak van pesticiden in biozuiveringssystemen

Cover picture:

Illustration of a biopurification matrix

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Contents

List of abbreviations and symbols

Symbols

Symbol	Description	Unit
$C_{l,eq}$	pesticide concentration in the liquid phase at equilibrium	mg L^{-1}
$C_{l,ini}$	initial pesticide concentration in the liquid phase	mg L^{-1}
$C_{l,ini}$	pesticide concentration in the liquid phase	mg L^{-1}
C*s*	sorbed concentration	mg kg^{-1}
$C_{s,eq}$	sorbed concentration at equilibrium	mg kg^{-1}
D	dispersion coefficient	cm^2 h^{-1}
K_1	Langmuir coefficient	g kg^{-1}
K_2	Langmuir coefficient	L kg^{-1}
K_d	distribution or sorption coefficient	L kg^{-1}
k_{decay}	decay rate of the biomass	h^{-1}
K_f	Freundlich coefficient	L kg^{-1}
$K_{f,batch}$	Freundlich coefficient in batch experiments	L kg^{-1}
$K_{f,column}$	Freundlich coefficient in column experiments	L kg^{-1}
K_{oc}	organic carbon partitiion coefficient	L kg^{-1}
K_{ow}	octanol-water partition coefficient	-
K_s	half saturation constant	mg_p L^{-1}
L	length	cm
M_l	amount of pesticide present in the liquid phase	mg
M_s	amount of pesticide adsorbed in the solid phase	mg
M_{sub}	weight of the substratum	kg
M_{tot}	total amount of pesticide	mg
n	Freundlich exponent	-
n_{batch}	Freundlich exponent in batch experiments (Chapter 4)	-
n_{column}	Freundlich exponent in column experiments (Chapter 4)	-
q	Darcian water flux	cm h^{-1}
q_{max}	high Darcian flux with pesticide-primed soil (chapter 6)	cm h^{-1}
q_{mid}	intermediate Darcian flux with pesticide-primed soil (chapter 6)	cm h^{-1}
$q_{mid+ref}$	intermediate Darcian flux with reference soil (chapter 6)	cm h^{-1}
q_{min}	lowest Darcian flux with pesticide-primed soil (chapter 6)	cm h^{-1}
R	retardation coefficient	-
R^2	determination coefficient	-
t	time	h or d
$t_{1/2}$	half-life	h or d

V	volume	L
X	pesticide degrading biomass concentration	$mg_b\ L^{-1}$
Y	yield coefficient	$mg_b\ mg_p^{-1}$
z	spatial coordinate	cm
α	first-order kinetic constant	h^{-1}
θ	volumetric water content	$cm^3_{water}\ cm^{-3}_{pores}$
λ	dispersivity	cm
v	pore water velocity	$cm\ h^{-1}$
μ^*	modified mass growth rate	$L\ mg_p^{-1}\ h^{-1}$
μ^*_m	modified mass growth rate	$L\ mg_b^{-1}\ h^{-1}$
μ_l	first-order degradation constant	h^{-1}
μ_m	mass growth rate	h^{-1}
ρ	bulk density	$g\ mL^{-1}$

Abbreviations

BTC	breakthrough curve
CDE	convection dispersion equation
DAD	diode array detection
DM	dry matter
GC	gas chromatography
GUS	groundwater ubiquity score
HDPE	high density polyethylene
HPLC	high performance liquid chromatography
LDF	linear driving force
LOD	limit of detection
MMO	minimal medium without any carbon source but with nitrogen containing salts
MS	mass spectrometry
OC	organic carbon
PV	pore volume
PVDF	polyvinylidene fluoride
SIM	single ion monitoring

Chapter 1: Introduction and general background

This chapter has been compiled from:

De Wilde, T., Spanoghe, P., Debaer, C., Ryckeboer, J., Springael, D., Jaeken, P. 2006. Overview of on-farm bioremediation systems to reduce the occurrence of point source contamination. Pest Management Science, 63, 111-128.

1.1 Pesticides as a miracle?

In a never-ending struggle to survive: man must eat. This need spawned the development and spread agriculture across the planet. As agricultural methods were refined and societies began to flourish, man became not just a feature and pawn of the environment, but was able to alter it in both useful and potentially harmful ways. As farming techniques have grown successively more sophisticated, the chemical industry provided, with the development of pesticides, solutions for the ever increasing need to increase quantity and quality of agricultural products. Another beneficial aspect of the use of pesticides is its important role as a suppressor of disease vectors and pests affecting the health and welfare of the world. Due to the high consumer expectations and the ever increasing world population, the use of pesticides seems insurmountable. In Flanders only, the quantity of pesticides used amounted up to 5 million kg active substance in 2005 (Milieurapport MIRA-T 2005).

The term *pesticide* will be used throughout this thesis and refers to synthetic organic plant protection products, which can be subdivided into insecticides, fungicides, herbicides, rodenticides, etc.

By their nature, pesticides are harmful to some forms of life. It is therefore not surprising that, at certain level of exposure, they may be harmful to humans. Occupational pesticide exposure has been linked to acute health effects including rashes, skin, eye, and respiratory illnesses, and death[1,2]. Chronic effects, including neurological, reproductive, pseudo estrogenic effects and cancer, are more difficult to ascertain, but studies have found associations between pesticide exposure and these effects[3].

When pesticides are applied under appropriate cropping and climatical conditions in prescribed amounts using specified procedures, they can be effective in pest control with little adverse effects on the surrounding environment. Measurements indicate, however, that trace amounts of pesticides are present on non-agricultural land, in the atmosphere, and in water. A vulnerable and important compartment of the environment is water. The contamination of water by pesticides is a major environmental issue in Europe[4-6]. Water covers about two-thirds of the Earth's surface, admittedly. But most is too salty, only 2.5% is not salty, and two-thirds is locked up in the icecaps and glaciers. Drinking water for human purposes is therefore limited to 0.08% of the earth's water. Contamination of these limited resources could be catastrophic.

Rivers, lakes and coastal waters are vital natural resources, they provide drinking water, crucial habitats for many different types of wildlife, and are an important resource for industry and recreation. A significant proportion of them are environmentally damaged or under threat partly by the use of pesticides. With substantial levels of pesticides now contaminating European water resources, drinking water companies across the EU are forced to spend large sums on water treatment every year. Estimates suggest annual investments of €24.4 million in the Netherlands, €130 million in Germany, and €170 million in the UK (www.pan-europe.info). Ultimately these costs are passed on to the consumer.

Quality standards for pesticide concentrations in drinking water are specified by the EU Directive and allow a maximum residue of 0.1 $\mu g\ L^{-1}$ for an active ingredient and of 0.5 $\mu g\ L^{-1}$ for the total pesticide load at the tap (98/83/EEC) (http://ec.europa.eu/environment /water/water-drink). The same maximum concentrations apply for groundwater. Plant protection products are already regulated in Europe under Directive 91/414/EEC (http://ec.europa.eu/food/plant/protection/). A revision of this directive in 2009 could lead to an exclusion of 75% of the active substances currently available. Moreover, there is an increasing trend towards additional regulations originating from other areas. The Water Framework Directive 2000/60/EC (http://ec.europa.eu/environment/water/water-framework) advocates an integrated and coordinated framework for the sustainable management of all surface and ground waters and requires them to be of 'good ecological' status by 2015. Thus, the control of pollutants is important in order to meet this demand[7].

A state of the art of groundwater contamination in 2006 in Flanders is given in Figure 1.1. The amount of red and purple dots indicate that the contamination of groundwater is significant and that mitigation measurements should be taken. As the groundwater is already filtered through the vadose zone, contamination of surface water will be much higher.

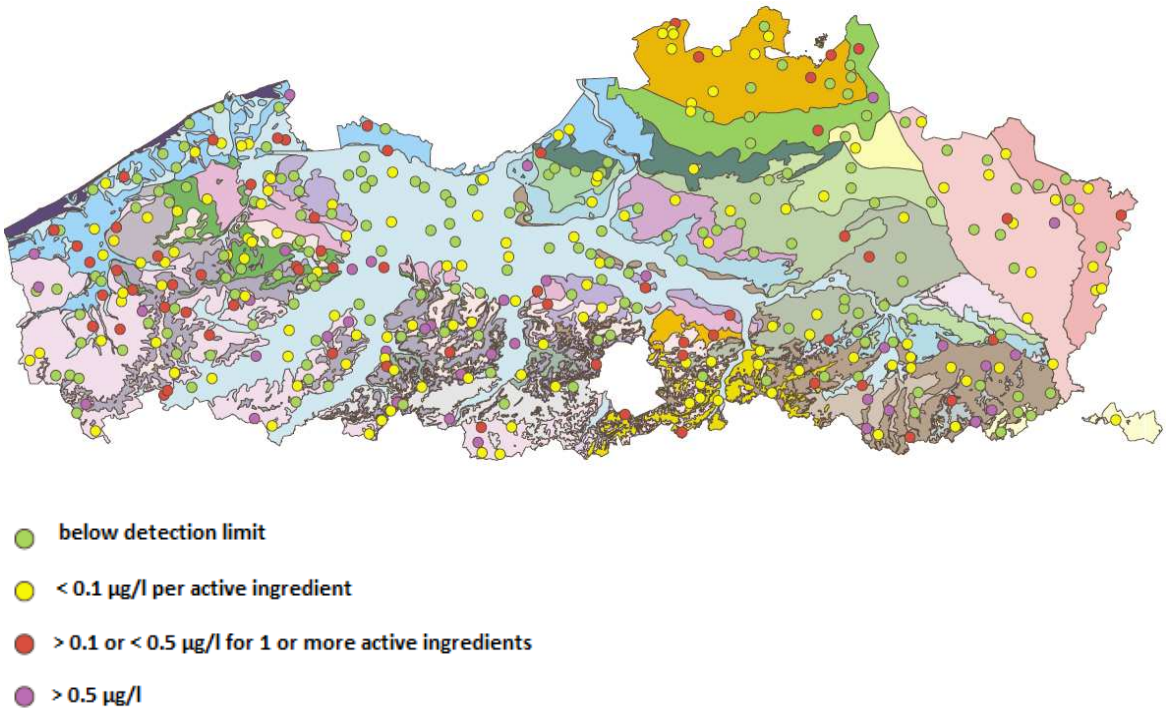


Figure 1.1: Distribution of pesticides in the freatic groundwater of Flanders (spring 2006) (Source: Milieurapport MIRA-T 2006)

1.2 How do pesticides enter the water?

Unsatisfactory management of pesticides and other chemicals can give rise to residues in surface and groundwater. One source of contamination is the use of pesticides in agriculture. Emissions of pesticides are generally subdivided into diffuse and point sources. Diffuse contamination via percolation, runoff, drainage and drift explains only a part of the applied pesticides that reach surface and groundwater. Several field surveys and measurement campaigns on catchment scale have demonstrated that 40 to 90% of surface water contamination by pesticides is attributed to direct losses[8-13]. The main direct losses are spillages resulting from the filling operation, leakages of the spray equipment, spray leftovers and technical rest volumes in the tank, pump and booms, rinsing water from cleaning the internal tank to avoid carry over effects (damage and residues) onto the following crop, water from external cleaning of spray equipment, etc.[14-17]. These point sources are in conclusion mainly linked to the filling and cleaning area on the farm. In Table 1.1 examples of point sources from mixing and loading facilities on farm sites in Wisconsin are presented[18]. The highest pesticide concentrations were found in areas of known spills, burn piles, mixing/loading areas and discarded pesticide container storage areas.

Table 1.1: Range of herbicide residues (mg L^{-1}) detected from specific use areas at 20 Wisconsin application facilities[18]

Sampling area	alachlor	atrazine	cyanazine	metolachlor
Mixing/loading	0.9-5900	0.1-3562	1.0-530	0.7-2730
Drainage way	0.1-86	0.1-454	0.1-285	0.2-927
Equipment parking	0.6-108	0.7-516	0.2-12	0.4-145
Acute spill	360-1940	8.1-750	15-190	0.5-75900
Discarded container storage	1.5-15	0.8-1090	2.0-341	1.0-1010
Burn piles	37-170	60-553	4.3-871	272-7720
Equipment washing	2.8	1.2-61	0.2-2.2	0.2-25
Weigh scale pit	01.7-59	0.9-1780	0.4-5.6	0.8-685
Pesticide storage	3.1-112	2.7-459	0.3-1.0	1.4-16.8

Sites for filling and loading are often covered with gravel and sand where the ability to sorb and degrade pesticides is low. In some regions these sites are often located near open wells and well borings in the farm area, which increases the potential of ground water contamination[19,20]. The impact of small quantities of concentrated spray solution is known to impose a serious contamination risk. A spill of a few milliliters of formulated preparation from a container can easily contain 1 *g* active ingredient. 10 000 m^3 water is needed to dissolve this amount to the acceptable concentration of 0.1 $\mu g\ L^{-1}$ water. Such point sources or direct releases may be reduced up to 95% by applying Best Management Practices and routines (www.voluntaryinitiative.org.uk). This is generally acknowledged in numerous stewardship campaigns (*e.g.* H_2Ok campaigns by national crop protection associations IVA (D) and CPA (UK) (www.voluntaryinitiative.org.uk), the stewardship project Nil (B) achieved a removal of 60-80% of the amount transported into the Nil in 3 years time [8]). However, some releases may still occur which makes it difficult to come up to the quality standard of drinking water of 0.1 $\mu g\ L^{-1}$ for an active ingredient. Therefore additional technological and infrastructural solutions are required to reduce these direct pesticide releases. A number of possible solutions are proposed including (a) the washing and rinsing of the spray equipment in the field[21-23], (b) a contained filling and loading place on the farmyard to minimize the release of pesticides[23,24], (c) waste water treatment systems that can separate and/or degrade the contaminants from the water fraction[25-27].

1.3 Physico-chemical waste water treatment systems

Various pesticide treatment systems exist or are under investigation. The practicality for on-farm implementation is heavily depended on investment and running cost and waste disposal cost. Many researchers have been seeking suitable methods to treat pesticide wastewater. Physico-chemical and biological methods are the main treatment process. Physico-chemical treatment can obtain a high efficiency and is stable with a high quality effluent, but the treatment cost is very high. Conventional treatment technologies including chemical coagulation, sedimentation, filtration, clarification, disinfection, have been widely used but they were not always successful. Pesticides which are insoluble in water, such as DDT, or which are easily decomposed, such as carbamate insecticides, generally can be removed effectively by the conventional methods. However, conventional treatment has often been proved ineffective for the separation of the majority of pesticides or their metabolites. Therefore, a number of innovative water treatment methods have been developed to create more efficient systems, particularly for the hydrophilic organic compounds[28]. The applied methods are classified in two categories. The first category, which includes adsorption and membrane technology methods, is based on the removal of toxic organic substances[29]. The second category of methods, such as oxidation, ozonation, voltammetry, photocatalysis is based on pesticide decomposition[30,31].

Adsorption/filtration is one of the well-known methods used in the removal of hazardous compounds from polluted water. Adsorptive or reactive filters contain a medium that adsorbs or reacts with a water contaminant. Activated carbon filtration is an adsorptive process in which the contaminant is attracted to and held (adsorbed) onto the surface of the carbon particles[32]. The efficiency of the adsorption process is influenced by carbon characteristics (particle and pore size, surface area, solubility of the contaminant, and contaminant attraction to the carbon surface). The medium for an activated carbon is typically petroleum coke, bituminous coal, lignite, wood products, coconut shell, etc. The carbon medium is activated by subjecting it to steam and high temperature without oxygen. A variety of activated carbon materials have been used, such as, granular activated carbon (GAC), powdered activated carbon (PAC), carbon cloth, fibers, felts or carbon cloth electrodes, black carbon from wheat residues (WC), carbon black and commercial activated carbon (AC). The forms GAC and PAC are the most used since they are considered very capable and effective materials for the adsorption of a variety of pesticides. The treatment of pesticide waste water with activated carbon was reviewed by Kyriakopoulos & Doulia[33].

Oxidation systems are based on the generation of highly reactive intermediates that initiate a sequence of reactions resulting in the destruction and removal of organic pollutants and are generally referred to as advanced oxidation processes (AOPs). These processes can be divided in three sections: (1) photochemical processes, (2) ozonation, and (3) technologies based on in situ generation of free hydroxyl radicals[31].

(1) Photochemical processes are light induced reactions, mainly oxidations, that rely on the generation of hydroxyl radicals by the combination with added oxidants or semiconductors, e.g. enhancement of the Fenton's reaction (a mixture of H_2O_2 and ferrous or ferric ions) by supplemented UV/visible irradiation.

(2) Ozone is a powerful oxidant, which is generally produced by an electric discharge method in the presence of air or oxygen. A disadvantage is however its expensive production.
(3) In-situ generation of radicals, e.g. electrochemical treatment and wet air oxidation.

An example of a physico-chemical on-farm treatment system of pesticide waste water is the Sentinel developed by E. Allman & Company, Ltd., UK (Figure 1.2). This installation is based on flocculation, sedimentation, sand-filtration and active carbon filtration. Firstly, flocculation chemicals are mixed with the waste water. Secondly, the formed sludge is sedimentated at the bottom of the tank. Finally, the resulting supernatant flows through the sand/gravel filter and both carbon filters, respectively. The sludge is removed after every five batches and after additional consolidation transported to an incinerator as chemical waste.

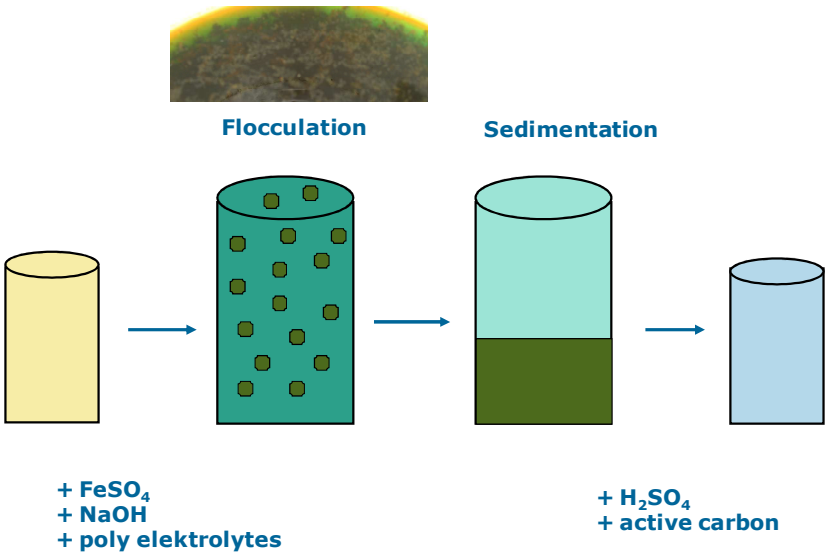


Figure 1.2: Principle of Sentinel physico-chemical treatment system

1.4 Biopurification systems

Waste water treatments discussed in 1.3 are very effective, but in most cases too expensive for average farmers[23]. Moreover the entrained pollutants (such as in the treatment with activated carbon) must be further treated for complete destruction. Therefore some form of biological processing is usually the preferred method for the treatment of effluents containing pesticides. For agricultural purpose these treatment systems need to be cheap and reliable, easy to use with low labor and time input and low waste disposal cost. A possible approach is the use of biopurification systems to capture and treat contaminated water from the farmyard and/or spillages from the filling process. Research has already proven that biopurification could be a low cost and efficient solution[23,34].

Bioremediation or biopurification is defined as the process in which organic wastes are biologically degraded under controlled conditions by microorganisms or their enzymes to an innocuous state, or to levels below concentration limits established by regulatory

authorities[35]. In its most simple form it uses naturally occurring bacteria and fungi or plants[36].

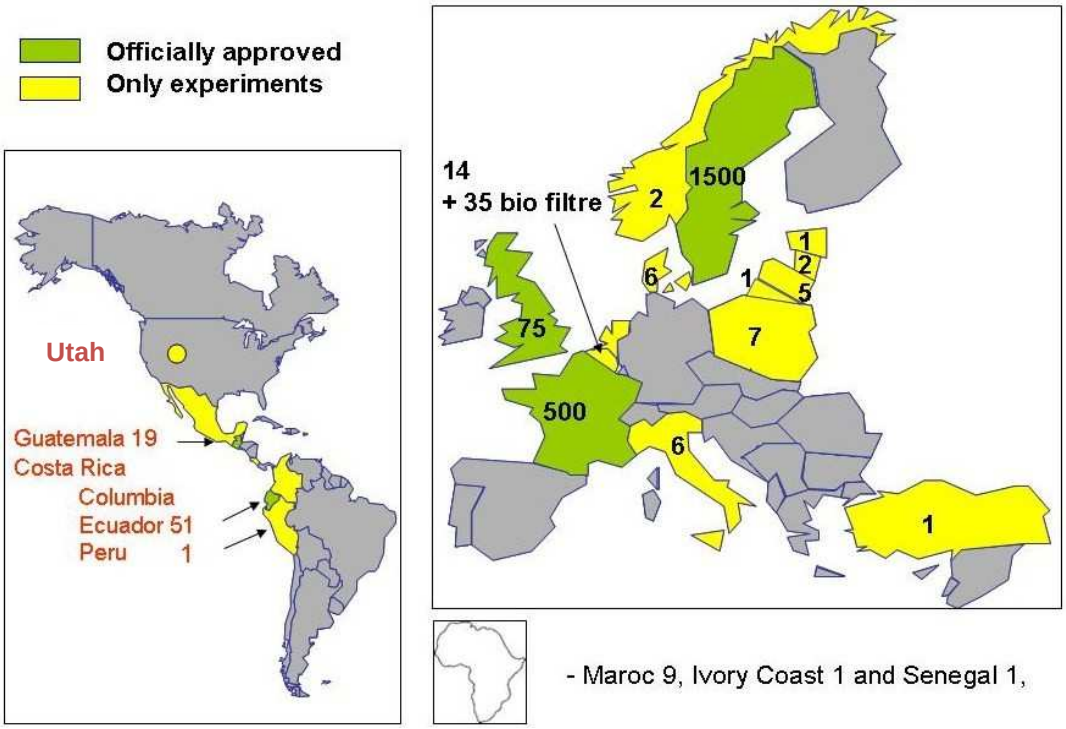


Figure 1.3: Distribution of biopurification systems over the world in 2008 (personal communication Jens Husby, Bayer Crop Science, Denmark)

Nowadays four main biological techniques for treating soil and groundwater are used: (a) stimulation of the activity of indigenous microorganisms (biostimulation) by the addition of nutrients, regulation of redox conditions, optimizing pH conditions, etc; (b) inoculation of the site by microorganisms with specific biotransforming abilities (bioaugmentation); (c) application of immobilized enzymes; and (d) use of plants (phytoremediation) to remove and/or transform pollutants[37].

The biopurification system has generated interest in various countries all over the world (Figure 1.3). Its implementations has sometimes led to modifications of the original Swedish design. The internationally best developed and tested biopurification systems for treating spray leftovers and pesticide spillages in agriculture are the biobed, the Phytobac© and the biofilter. The concept of these three systems is similar. They all consist of a biological active matrix which retains the pesticides onto organic matter or soil particles, where enhanced or rapid microbial degradation of the pesticides occurs.

1.4.1 Operational context

Biopurification systems are in many cases easy to construct by farmers. It is flexible for adaptation to local conditions including both farm specific and geoclimatic variables. Superimposed on this situation is the impact of the local legislation, mainly on waste disposal options. This situation results in a wide variability and differentiation of biopurification design and functional components. Figure 1.4 shows a simplified flow process with back coupling mechanisms.

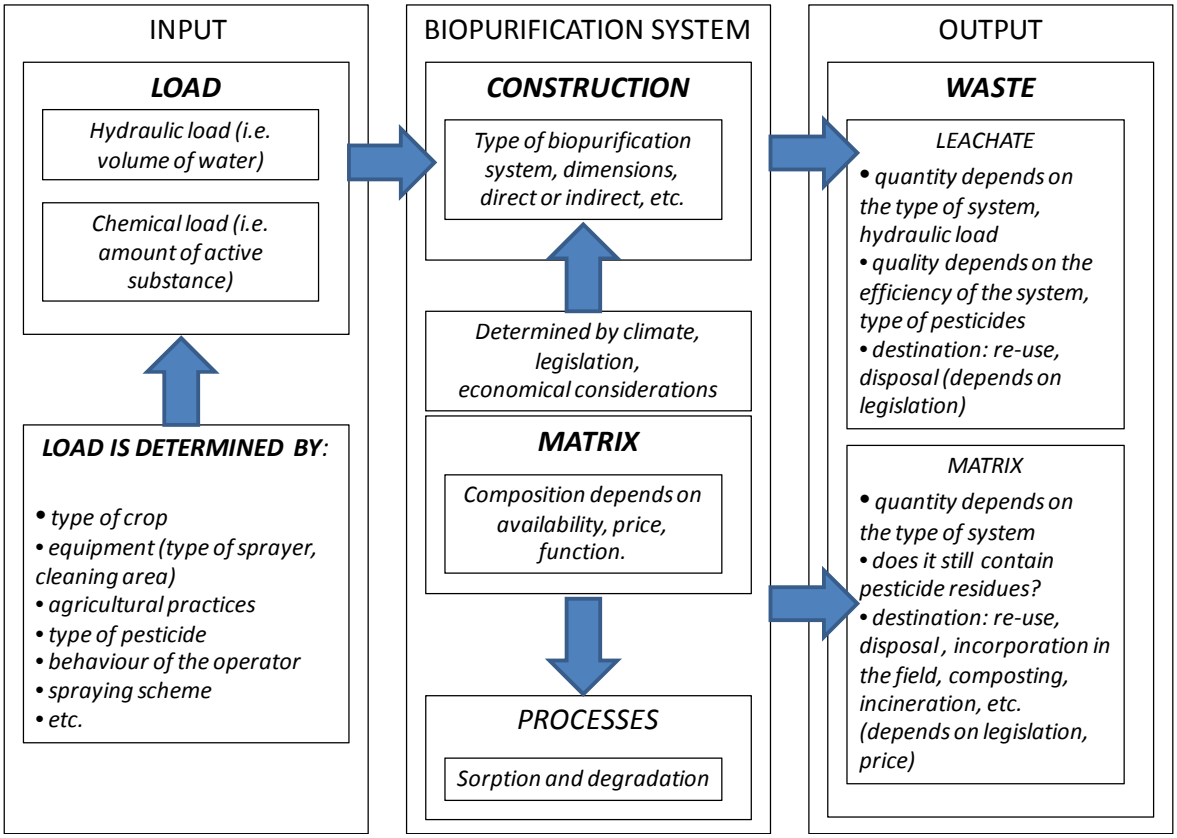


Figure 1.4: Schematic overview of the operational context of biopurification systems

For most biopurification systems the scheme in Figure 1.4 provides a general framework which will determine the design and dimensions of a system depending on the farm or local specific situations. Under all conditions, it is obvious practice to minimize hydraulic and chemical input by avoiding unnecessary rinsing, applying good cleaning practice and preventing clean rainwater to enter the system. At the output side of the system, liquid and solid waste should be minimized by proper dimensioning according to the needs. In most EU countries the policy towards this type of waste and its destination is still not established.

Hence the total cost of any given system (investment, running and waste disposal cost) and the opportunity cost of other end of the pipe solutions or preventive measures to manage point source risks is still topic of debate.

For biopurification systems, the quantity and frequency of hydraulic and chemical load, the climate, and legislation will have an impact on the type of design and the quantity of matrix

substratum. All these factors have to be considered in order to come to the most suitable biopurification system for any given farm or application.

1.4.2 Active matrix or substratum

1.4.2.1 General conditions for biopurification

The control and optimization of biopurification processes is complex and depends on many factors. These factors include the existence of a microbial community capable of degrading the pollutants, the availability of contaminants to the microbial community, and environmental factors like type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients[36].

To solve the problem of no or low microbial activity, the nutrient limitations of carbon or nitrogen must be counteracted by providing nutrients in order to stimulate growth and activity of microorganisms. Another major constraint for biodegradation is the accessibility of the pesticides for microorganisms, *i.e.* the bioavailability[38]. The bioavailability of a chemical in soil is influenced by a number of factors, including concentration, type and quantity of clay and organic matter, pH, temperature, and the compounds chemical characteristics[39]. The current understanding of bioavailability suggests that, if the aqueous concentration of a pollutant is increased (*i.e.* partitioning from the solid to the aqueous phase), a greater contact between the microbe and the pollutant can be attained, thereby increasing the pollutants bioavailability[38]. The biodegradation of an organic compound is almost exclusively situated where pollutants are dissolved in the soil water phase that surrounds the microorganisms. In other words, it is within the thin water layer on the surface of a substratum where the microorganisms are situated, *i.e.* the biofilm, that biodegradation will take place. Therefore increasing the specific surface of a substratum without losing bioavailability due to micropores (clay) will provide more binding sites for biodegradation by microorganisms.

1.4.2.2 Materials used for the active matrix or substratum

The first reported substratum mix used in bioremediation systems consisted of peat, straw and topsoil. These compounds were originally mixed in volumetric proportions of 1:2:1 respectively, and is called biomix[34], further referred to as “original biomix”. Peat provides numerous sites for pesticide sorption. It helps to maintain aerobic conditions combined with sufficient humidity or moist due to its high water-holding capacity and regulates pH. High amounts of peat are not recommended because of its negative effect on phenoloxidase and respiration activities[40]. Peat is also contested as a non-sustainable raw material. In existing bioremediation systems peat is often replaced by different types of compost. Compost and peat also have the function to ensure the presence of a variety of microbial strains. However, as peat regulates pH of the biomix, the replacement of this substratum can increase pH which reduces the phenoloxidase activity and might increase mobility of certain pesticides (*e.g.* in case of some sulfonylurea pesticides)

Straw acts as an additional food source for the microorganisms. It provides lignin for lignin-degrading microorganisms, which produce enzymes (*e.g.* phenoloxidases) catalyzing the degradation of a broad spectrum of chemicals[41]. Torstensson and Castillo[16] investigated the capacity of different mixtures of topsoil-peat-straw to degrade pesticides. They found

that when the straw fraction increased, the respiration and thus the microbial activity increased. This resulted in an increased degradation of bentazone, chloridazon and linuron and hence a better performance of the biopurification system (Figure 1.5). This is however in contrast with the results of Coppola *et al.*[42] who did not observe significant differences in the dissipation and mineralization rates of chlorpyrifos at different straw fractions. For practical reasons, not more than 50% straw can be used.

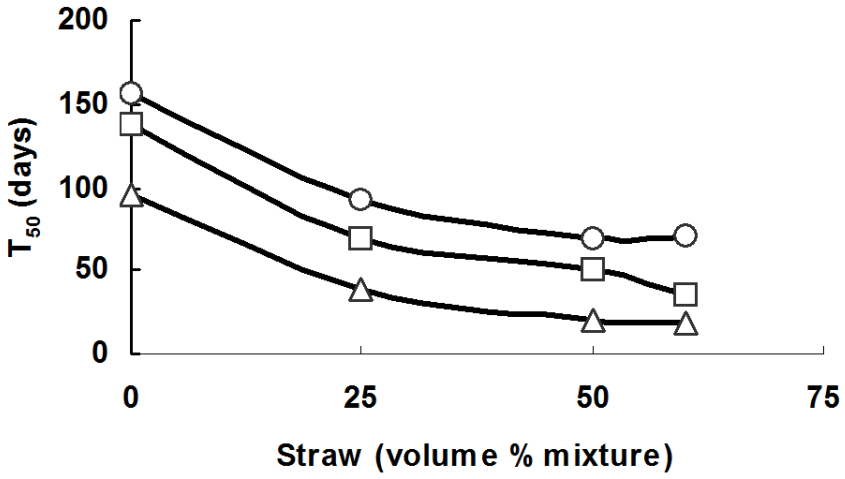


Figure 1.5: Influence of various amounts of straw added to a biobed containing top soil and peat on the half-life ($t_{1/2}$) of three herbicides (Δ) bentazone. (○) chloridazon and (□) linuron [17]

The topsoil acts as an inoculum of microorganisms for the biomix and should be rich in humus but should have a low clay content[16]. As biopurification systems are built using locally available materials, it is obvious that the physical and chemical characteristics will vary considerably from system to system. There is evidence that soil texture influences the rate at which pesticides degrade[43,44]. Furthermore, if a considerable soil fraction is included in the substratum mix, the soil texture is dominant in the water movement control. Leaching of pesticides is typically associated with low organic matter content, low moisture-holding capacity, and a relatively sandy texture[45]. However, the contrasting characteristics of the different topsoil textures may not be as relevant in the biopurification matrix as for *in situ* soils due to the destructive mixing process, the limited amount of soil applied (maximum 25%) and the inclusion of peat and straw[46]. The study of Fogg *et al.*[46] showed that when different topsoils were used in the biomix, leaching losses and degradation rates were similar. More than 98% of the applied pesticide was retained for each of the biomix types. Therefore the use of different soil types in the construction of the biobed seems to have less influence on biopurification. These results are confirmed by De Vleeschouwer *et al.*[47], who observed no significant difference between a clay soil and a silt soil. Based on this knowledge, it should be possible to use local soils in the construction of each system. Local pesticide treated field soils might contain naturally occurring and potentially pesticide adapted microbiological populations. The surplus value of a biomix compared to topsoil was only studied by Fogg *et al.*[48]. A faster degradation for 6 out of 7 investigated pesticides was found in a biomix compared to topsoil.

Various studies investigate the use of alternative substratums. Starting from the original biomix, various primary substratums have been mixed for biopurification purposes, both in laboratory studies and in pilot installations. In Italy, agricultural waste by-products were used[49]. Investigated substratums were vine branch, citrus peel, green waste compost and urban waste compost. Vine branches which had a higher organic carbon content were expected to sorb the pesticide better compared to citrus peel. However, the opposite was observed. This could be related to the different size of the two organic materials. Vine branch is a coarse material, with a specific surface that is lower than that of citrus pulp. Adsorption was in this case more related to the specific surface than to the organic carbon content. Green waste compost was less efficient than urban waste compost. However, no comparison was made with the classical biomix composition. Thus one can not state that the implementation of these substratums will be more or less efficient than the original composition.

Genot *et al.*[50] studied a mixture of substratums in another biopurification system, the biofilter, which is discussed in 1.5.1. The highest retention of pesticides was found in a soil-manure mixture, followed by soil-chitin and then by soil-straw. Degradation was higher in the soil-manure than in the soil-straw mixture. This is in contrast to Torstensson and Castillo [16,34] who found a slower degradation of isoproturon and bromuconazol in a manure-based mixture of a biobed system. According to Torstensson and Castillo[16], this was caused by the absence of readily metabolisable organic matter in aged manure. A comparison between manure and green waste compost was made by De Vleeschouwer *et al.*[47]. It appeared that manure mix had a higher efficiency (= the capacity to reduce the loss of active substances in the effluent of the biofilter) and resulted in a higher pesticide degradation rate than the green waste compost. When peat and green waste compost were compared, it was found that the green waste compost was less efficient and that the degradation rate of this substratum was slower compared to peat. Concerning the use of straw, a comparison was made between flax straw and grain straw. Flax straw appeared to be better performing on the level of efficiency and degradation of persistent molecules[47]. Reports on pilot installations include coco nut waste products (peat fibre, crushed shell and chips)[51].

Recycling the substratum of the biofilter after one year by mixing it with fresh organic material, such as straw or manure, was found to improve significantly the performance of the biofilter[50]. Although the presence of recycled material improved retention of the pesticide in the mixture, it did not improve degradation. Therefore the improvements of efficiency observed were at least partly caused by a more homogeneous structure of substratums. As after a few years, the material will deteriorate, Torstensson[52] recommended to completely change the substratum of the biofilter. However Genot *et al.*[50] showed that it would be more beneficial to remove only a part of the material and to mix the rest with fresh organic material. This improves the structure, and the microflora adapted to the substratum can colonize the fresh material.

1.4.3 Composting of active matrix or substratum

The total content of carbon, the ratio between carbon and nitrogen, and the microbial activity decreases the longer the biopurification system is in use[52]. When the rate of the basal respiration (indicator of microbial activity) in the biobed reaches the same value as the basal respiration in the soil, the material in the biopurification system should be renewed.

This takes 5-6 years in the south of Sweden[52]. The lifetime of the biobed depends on how often the biobeds have been filled up after initiation. The material will shrink about 10 cm a year for the biomix. This shrinking level will however depend on the material used. Farmers are recommended to remove the grass cover from their biobeds and to fill up their beds with new straw-peat-soil mixture every year. The recommended time to change the biomix is in springtime, just before the new spraying season as at that time the lowest concentrations of pesticide residues are found[52].

After removal, the biomix material should be transferred to a safe location on an impermeable medium (to avoid leaching) where it may compost for a further year to allow further degradation. During that year it is beneficial to turn or mix the material to ensure maximum degradation of remaining pesticides. In a study of Torstensson[52], pesticides were analyzed in four biobed systems. It was seen that after one year, only one biobed still contained small amounts of pesticides. The disposal of the biobed material will be required to comply with the waste regulation[53]. To dispose the substratum after use, it is essential that the pesticide residues that are retained within the biomix are degraded and not simply retained within the organic matrix of the system. Fogg *et al.*[48] performed experiments in sterile and non-sterile soil with chlorothalonil, in order to determine whether the decline in observed residues originated from degradation or sorption to the matrix. Degradation appeared to be minimal in the sterile biomix compared to the non-sterile one (Figure 1.6). Therefore they concluded that degradation was the main process responsible for the removal in residues. Irreversible binding to the biobed mixture was of minor importance.

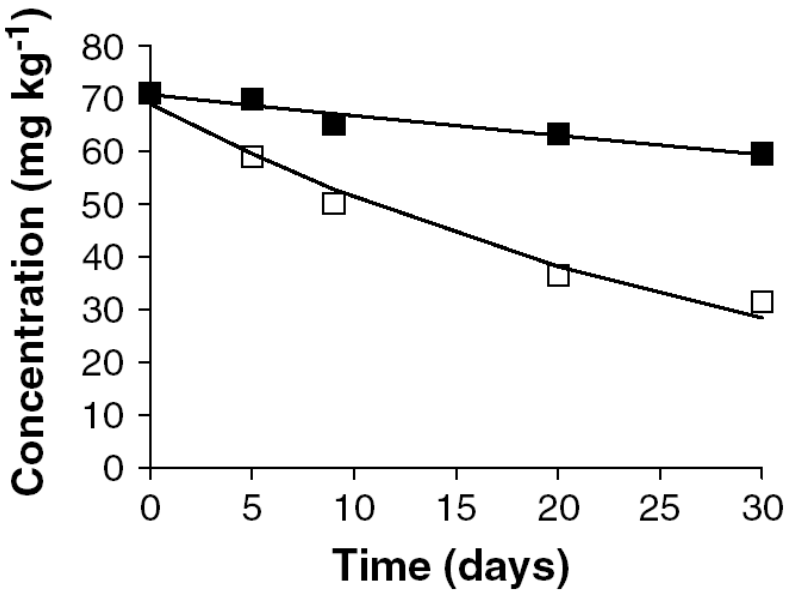


Figure 1.6: Degradation of chlorothalonil in (■) an autoclaved 'sterile' biomix and (□) in a biomix that has been autoclaved and re-inoculated with a non-sterile biomix[48]

1.5 Biopurification systems: design, operation and functionality

Prior to review the three main biopurification systems, two distinct aspects are highlighted: the collection and the outlet construction. The collection construction can be drive over, off set or direct sprayer discharge. A drive over construction allows the sprayer-tractor combination to be positioned on top of the biopurification system. Liquid basically falls on the system by gravity. Any co-pollutants like engine oil and grease are difficult to keep out of the system. An offset system separates the collector site from the biopurification system. It allows an oil separator to be positioned in between collector and remediation part. Often the waste water is homogeneously distributed over the system from an intermediate storage tank. The direct sprayer discharge, uses the hydraulic system of the sprayer to pump it directly on the biopurification system. This makes external cleaning in the field imperative and excludes accidental spills to enter the system. On the other hand it eliminates mud and other substances to enter the system and minimizes operator exposure.

The outlet construction offers various options: it can be unlined, semi-permeable lining and impermeable lining. Unlined means an excavated hole in the soil, filled with a biomix. The boundary zone under it is the remaining part of the original soil horizon. Since not all *in situ* soil profiles and soil horizons have proper soil texture properties, semi-permeable lining of the outer surfaces is often executed with a clay layer. Impermeable lining does not allow water to leave the system by means of percolation. Impermeable lining can be used for a completely closed system or for a controlled drain system. The latter allows re-pumping of leachate to the biopurification part or to pump it to a following clean up or leachate removal step like *e.g.* phytoremediation.

1.5.1 Biobed

Sweden introduced the biobed to handle spillages of pesticides in connection with filling of sprayers[16]. The first biobeds were built in 1993 according to the design by Torstensson and Castillo[54]. Today there are more than 1000 biobeds in practical use in Sweden[20,52]. In its simplest form, a biobed, situated at the filling place, is a clay-lined hole in the ground filled with the original biomix, which, as mentioned, consists of peat, soil and straw. The biomix should be left for some 5-8 weeks prior to loading in the biobed pit[55,56]. By this time, the composting operation has begun and the mix is likely to be effective at retaining and degrading the pesticide residues. The biomix is covered with a grass or a turf layer. The grass layer maintains an optimal level of temperature for microbial activity, regulates the moisture in the bed and serves as an indicator of pesticide spillage[16,34]. In Figure 1.7 a general spillage pattern on a biobed is presented[52]. From this graph it can be concluded that a lot of pesticide wastes are generated under the spraying tank. It also shows the poor primary distribution of the chemical loading on the system. The turf layer ensures that there is good rooting activity. It assists in moisture management, through evapotranspiration of water to the atmosphere[53]. This layer also reduces the amount of excess precipitation and therefore reduces leaching[57].

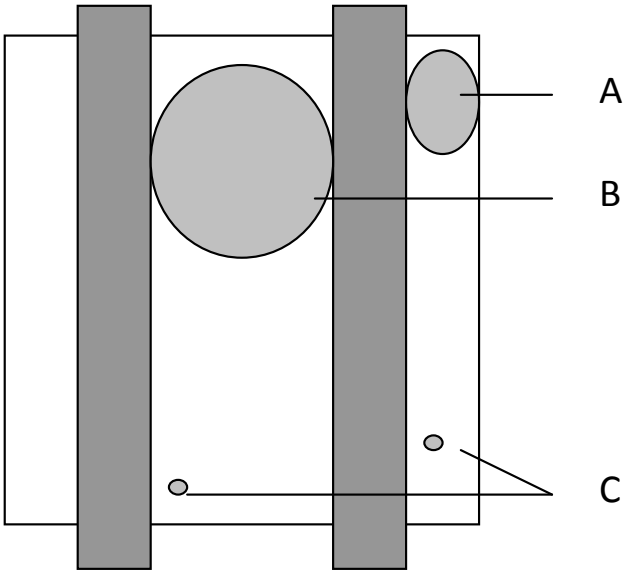


Figure 1.7: General spillage pattern on a biobed: A. spillage due to preparation of containers B. Pesticides which adhere to the outside of the sprayer tank are washed off by spillage of water during the next filling process or by rainwater when the sprayer is parked on the biobed C. Dripping nozzles[52]

A biobed is equipped with a ramp supporting the tractor and sprayer to be driven over the bed and thus enabling the biobed to intercept drips and spills (Figure 1.8). All liquid inputs (rainfall and pesticides) enter the biobed exactly where they fall and therefore they are not equally distributed. Studies performed in Denmark have shown that the clay membrane at the base of the biobed could not retain all of the leachate draining through the biobed[58]. Studies have also shown that whilst less mobile pesticides are effectively retained within the biobed matrix, significant amounts of the more mobile pesticides can leach from the biobed through the clay layer[57-59]. Fogg *et al.*[60] investigated two systems in the UK: a lined system where the biomix was enclosed in a sealed column and an unlined system where leachate was able to percolate through the bottom of the biomix. The use of a lined system was considered attractive to regulatory authorities as it minimises the potential for leachate to contaminate groundwater. Studies with lined biobeds demonstrated that pesticides displaying a range of physico-chemical properties were effectively retained. However, some disadvantages could be found. Monitoring of soil moisture status indicated that lined biobeds needed to be covered in order to exclude rainwater from the system. Once covered, the surface layer (0-10 *cm*) rapidly dries out and forms a hydrophobic layer, severely restricting evaporation and thus moisture loss. This resulted within 12 months after construction in water saturated conditions below 10 *cm* depth as evaporation is hindered through the hydrophobic layer. The dehydration of the surface layer was also associated with a decrease in the microbial biomass. Therefore, covering of the system may not be a practical solution. In contrast, unlined biobed columns, which are uncovered, allow the water to leach, thus avoiding any need to manage water inputs. Unlined biobeds also showed positive results. From the six pesticides tested on the unlined biobed only two leached, and even for these two pesticides, more than 95% was retained.

Figure 1.8: Example of a biobed

Too much water causes a faster leaching of the pesticide, and therefore the location of the biobed on the farmyard should be carefully considered to prevent water flowing in. They should *e.g.* not be located close to a building with a roof without a drain and not at the lowest point at the farmyard, were water can easily enter the biobed. Furthermore it should also be avoided to place biobeds within a barn. This results in poor growth of the covering grass and causes very low evaporation, which results in a wet biobed.

The biobed is a cheap and practical solution. Boundary conditions for use are strict and it is intended for low chemical loading (www.odlingibalans.com). Main risks of drive over types are co-pollution from machinery oil and grease. Once chemicals break through the bottom clay layer at around 60 cm depth, soil degrading conditions will be unfavorable.

Due to the inexpensiveness, simplicity and efficiency, biobeds are also suitable for application in developing countries. One important difference is the often smaller size of farms in developing countries. Therefore, a small biobed for a person standing and filling a backpack sprayer is often sufficient[61].

1.5.2 Phytobac© or Biobac©

Very little has been published on this type of biopurification system, though books of charge for its construction in practice exists. In France, the agrochemical company Rhône-Poulenc Agro France took the initiative to experiment with the Phytobac© system. The Phytobac© is based on the biobed concept and is similar to the lined biobed described by Basford *et al.*[55]. The difference with an unlined biobed is that it is watertight. It is a cistern made from concrete or plastic foil and eliminates water only through evaporation. As a result, Phytobacs© present more flexibility in the overall design of the system. For instance, it is possible to recuperate rinsing water from the floor of a nearby located filling (and cleaning) area of the sprayer. This was not possible with an unlined biobed, due to the leaching of pesticides through the clay layer when a high hydraulic load was applied. In practice two different constructions have been presented. In an indirect system (Figure 1.9B), the Phytobac© is connected to an adjacent concrete intercept area on which all mixing and wash down activities (cleaning of the spray tank) take place. This system is mainly used in the UK[9,62]. Any liquid falling on the intercept area can be stored in a temporary storage

tank. The presence of such a tank allows much greater flexibility with regard to the rate and timing at which the liquid is discharged to the biopurification system. To maximise the potential surface area for pesticide retention and/or degradation processes to take place, the liquid from the temporary storage tank is evenly distributed over the entire Phytobac© surface by means of a drip irrigation system. The advantage of this system is that it can handle larger volumes of pesticide waste water. The second system is a direct system, where the Phytobac© is below the spraying boom (Figure 1.9A).

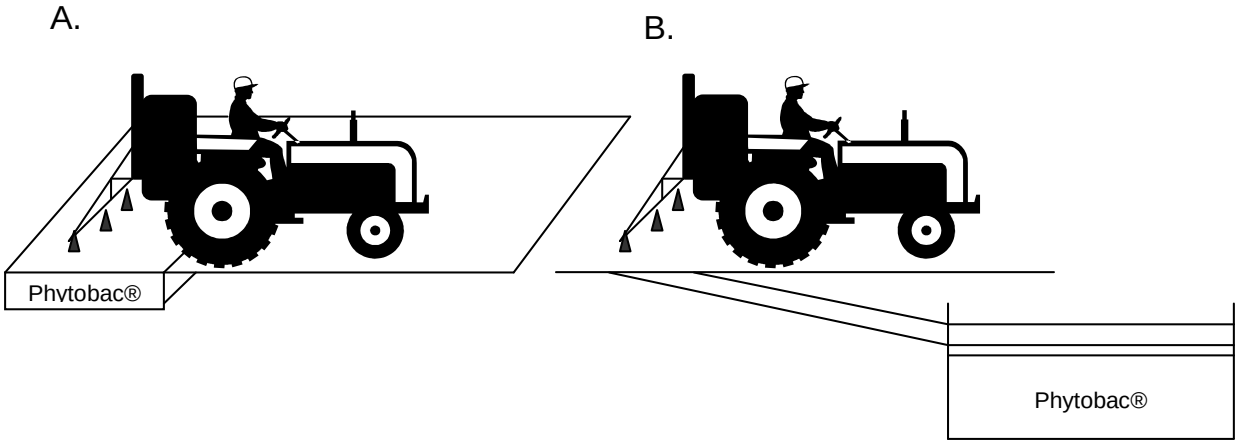


Figure 1.9: A schematic representation of a direct (A) and an indirect (B) Phytobac©.

Phytobacs© are generally large installations because poor water evaporation can be expected (Figure 1.10). Large volumes of substratum are needed in order to avoid complete saturation or even flooding of the substratum. Because evaporation will be enhanced and therefore run over will be avoided, the Phytobac© is most suitable for countries with a warm climate. Furthermore, in countries with a warm climate the surface of the Phytobac© can be reduced in contrast to countries with a colder climate, where a large surface is needed to obtain a similar level of evaporation. The Phytobac© should be covered to reduce the input of rain water, but because of the need for evaporation it should not be closed. To ensure that no dehydration or saturation occurs, the moisture content should be carefully controlled. To solve the problem of over saturation in a direct system, drains can be installed in the Phytobac© to collect the water in a buffer tank. This makes it possible to recycle the waste water several times over the system. This should improve the retention and degradation capacity[63].

Figure 1.10: Example of a Biobac© (www.biotisa.com)

The Phytobac© material is or can be similar to the substratums mentioned in previous sections with a good porosity for water and air (*i.e.* soil, peat), easily degradable organic material (*i.e.* straw, manure, green compost) and an activator such as compost to accelerate the degradation.

The results up to now are promising. The study performed by Fournier[64] showed that a small scale Phytobac© was very efficient to degrade 14 pesticides. The amount of pesticide residues present in the biomix after 2 years was not more than 1% for all pesticides, except for terbutylazine, for which 2.3% was detected (Table 1.2)[64]. A pilot installation in Belgium studied 7 pesticides[59]. After one year, almost no pesticide leached out. Furthermore, degradation must have occurred, because no detectable pesticides were still left in the Phytobac© substratum.

Besides the advantages of the Phytobac©**,** evaluation of the use at farmers' level showed that there are some drawbacks related to the Phytobac© system *i.e.* (1) difficult protection of the Phytobac© from rainfall; (2) possibility of clogging of the pipe circuit; (3) homogenization of the substratum was difficult to obtain, because mixing of the substratum was not easy; 4) although it is suitable to collect the majority of effluents, the capacity for receiving high water volumes was limited.

Table 1.2: Residues present in a 200 L bin filled with a mixture of 100 L soil and 100 L wheat straw after two year[64]

Pesticide	Type	Input (*g bin*$^{-1}$)	Residue after 2 year in the substratum (%)
atrazine	Herbicide	2.0	0.0
carbetamine	Herbicide	5.5	0.0
chloridazon	Herbicide	21.5	0.02
chlorpropham	Sprout inhibitor	2.1	0.0
diuron	Herbicide	16.2	0.01
ethofumesate	Herbicide	5.0	0.52
gluphosinate	Herbicide	1.5	0.0
glyphosate	Herbicide	16.4	0.14
isoproturon	Herbicide	10.0	0.0
isoxaben	Herbicide	4.0	0.0
metsulphuron-methyl	Herbicide	0.2	0.0
mesosulphuron-methyl	Herbicide	0.15	0.0
fenmedipham	Herbicide	1.6	0.0
terbutylazine	Herbicide	16.2	2.3

1.5.3 Biofilter

Biofilter is a general term in house hold waste water treatment systems. It is a group name for very different systems ranging from biorotor to active slurry systems. Originally a biofilter was another variation of the biobed, which was developed in Belgium. It consists of a serial number of subsystems (typically 2 or 3 units) depending on the hydraulic load. Each unit is made from a 1 m^3 plastic container or IBC (Intermediate Bulk Container). At the bottom, the containers have an internal drain towards an outflow valve to limit leftovers in the container. The different units are stacked in a vertical pile and connected with valves and pipes[65] (Figure 1.11). When comparing a two-unit biofilter with a three-unit biofilter, it could be stated that both biofilters performed very well. Books of charge for practice recommend a two-unit biofilter when the volume of effluent is less than 3000 *L* a year and when the total chemical load in the waste water is less or in the range of 100 *g* of active ingredient. For higher volumes of effluents and higher loads, it is recommended to switch to a system with three filtering units[65].

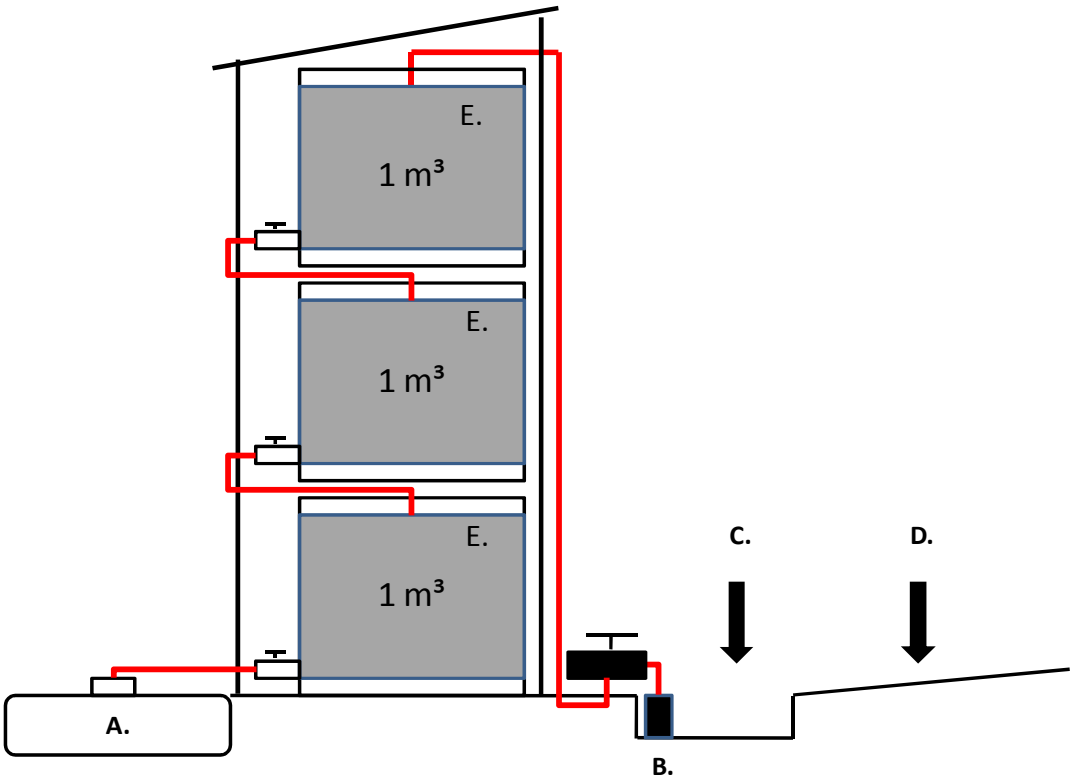


Figure 1.11: Schematic representation of the biofilter design. A. Slurry put, B. Suction pump, C. Sink for collecting waste water, D. Area for filling and cleaning the sprayer, E. Mixture containing biomix[65]

A biofilter is able to treat large volumes of effluent containing low concentrations of active ingredients or small volumes of highly concentrated effluent. A container is filled with adsorbing materials such as soil, peat and straw. The water percolates through the material and leaves the biofilter through a tap at the bottom of the tank[59]. A biofilter offers the advantage of combining the positive aspects of both biobed and Phytobac©. It is adapted to the treatment of large volumes of effluents (through filtration) and it offers a broad flexibility in the design of the system[51].

Monitoring of pesticides brought on the biofilter was carried out by Pussemier *et al.*[65]. For this study, five tracer pesticides were followed. The percentage of retention was generally higher than 95% in the biofilter material. All tracer pesticides, except lenacil, could not be detected after one year in the substratum. A study reported by De Vleeschouwer *et al.*[47], pointed out that in 20 different biofilter installations, the efficiency was higher than 92%. For 17 out of 20 biofilters it was even higher than 96%. The least water soluble pesticides, such as metconazole and iprodione were easily adsorbed on the organic substratum and therefore did not pose problems of leaching. More water soluble pesticides, such as carbofuran and chloridazon were less adsorbed and leached more easily through the substratum.

Concerning degradation, for 9 out of 15 pesticides 75% of the biofilters showed degradation of the pesticide higher than 95%. A lower degradation was observed for persistent pesticides such as lenacil, metolachlor, ethofumesate, azoxystrobine and metconazol.

A variation of the biofilter concept includes the growth of plants on the containers[51]. This can be beneficial for several reasons. It facilitates evapotranspiration and it can function as a bio-indicator and temperature regulator. The use of plants can also assist in stimulating biodegradation – phytoremediation by releasing inducers and enzymes that stimulate microorganisms, stimulate co-metabolic transformations by microorganisms, and transform organic compounds themselves. The disadvantage is that it does not allow a yearly manipulation of some subsystems of the biofilter. Substratum composition must be adapted to this situation. Furthermore, plant communities should be adapted to hydraulic load and herbicide use[51]. Phytoremediation can be applied to any lined system as a secondary clean up and removal step. Results of efficiency of such a system are in survey.

Biofilter systems are moderate in cost and have a favorable treated water/matrix ratio. Major risk on reduced efficiency is the relatively high surface/volume ratio of the subsystems, making it susceptible for poor packing and preferential flow in general.

1.6 Factors influencing the behaviour of pesticides in biopurification systems

The pesticides whose behavior has been studied on a biopurification system or in a biomix are listed in Annex. Those studies have been mainly performed by research groups in Sweden, Denmark, Italy and the UK. In total the behavior of 38 pesticides has been reported. Pesticides studied on a small scale Phytobac© are listed in Table 1.3.

A comparison between the different results is very difficult, due to high variability in influencing factors, such as the applied concentration, a different construction of the system, depth, application of pesticide mixtures, hydraulic loading, repeated applications, lifetime of the biomix, etc. Overall, it can be concluded that for most of the pesticides, more than 95% was degraded after one year. Therefore, looking at the continuation of the spraying season, no risk for accumulation is present. The huge range of systems, operating conditions and pesticides monitored clearly indicates the robustness of the system. Factors influencing the behavior of pesticides in the biopurification systems will be discussed.

1.6.1 Effect of pesticide concentration

Fogg *et al.*[66] investigated the influence of increasing pesticide concentrations on the degradation rate in topsoil and in original biomix. At concentrations ranging from half to 20 times the maximum recommended application rate for isoproturon and chlorothalonil, the rate of degradation decreased with increasing concentration (Annex– parameter: influence of concentration). This was explained by the suppression of the microbial activity. The same results were found by Henriksen *et al.*[57] for a biobed system loaded with isoproturon and mecoprop and by Vischetti *et al.*[67] for degradation of chlorpyrifos. To avoid high concentrations of pesticides on the biobed, internal cleaning of the spraying equipment with water from a freshwater tank should be performed in the field after application[57].

1.6.2 Effect of pesticide mixtures

In practice, biobeds are likely to receive complex mixtures of more than one active substance, often applied repeatedly[68-70]. The effect of the application of a mixture of chlorothalonil and isoproturon on biobed performance was studied by Fogg *et al.*[66]. Degradation of isoproturon was inhibited in the presence of chlorothalonil in topsoil, while it was not inhibited in the biomix (Annex– parameter: influence of a mixture). The results suggested that biobeds are capable to treat high concentrations of a combination of pesticides. Other studies which have been performed with a mixture of six active substances showed that, with the exception of epiconazole, degradation of the compounds applied to the biomix as a mixture was slower than when the compounds were applied individually (Annex– parameter: influence of a mixture). This inhibition was probably caused by a suppression of the microbial activity[37,71]. It is obvious that the problem of combined effects is very complex and cannot be studied in all possible combinations. Possible interactions can for the moment only be studied *ad hoc*.

1.6.3 Moisture Content and Hydraulic Loading

The moisture content in a biopurification system is important. It should be sufficiently high to allow activity of the pesticide-degrading microorganisms, but not so high that leakage of pesticides becomes a risk and that anaerobic conditions are created. A moisture content or water filled porosity of 100% of the biomix (± 0 *kPa* of suction) represents an oversaturated situation, with the risk of downward transport of pesticides. A water content between 95-100% of the biomix (20-60 *kPa* of suction) is required for adequate aeration of plant roots and represents a range for optimal microbial activity. Below 75%, moisture is insufficient for plant growth and is also limiting for microbial activities[52]. Castillo *et al.*[40] also tested the effect of three moisture levels (30, 60, and 90% of water holding capacity (WHC)) in laboratory biobeds. Moisture at 60% of WHC gave the highest dissipation of most of the pesticides tested, while moisture at 30 and 90% of WHC limited the microbial activity. Covering the biobed results into dry conditions in the top 10 *cm* and in the formation of an impermeable cap. This also causes a decrease in the biomass and degradation efficiency[72,73]. Adequate water availability is essential for microbial activity and thus for biodegradation. Experiments have generally shown an increase in the rate of pesticide removal with increase in soil moisture[71].

Biobeds in Sweden have been mainly installed to treat small volume drips and spills of pesticides originating from the spray fill site. However, if the system has to treat dilute pesticide waste and equipment washings as in the UK, it must cope with large volumes of rest and cleaning waters. In the study of Fogg *et al.*[74] three different hydraulic loadings were tested: direct rainfall (486 $L\ m^{-2}$), 2797 $L\ m^{-2}$ and 9747 $L\ m^{-2}$. Pesticide leaching was clearly affected by the hydraulic loading (Annex – parameter: influence of water loading). Increasing the water input on the biobed, resulted into increased leaching (Table 1.3). By controlling the water inputs and increasing the retention time within the biobed by increasing the depth, less leaching will occur for mobile pesticides. An increase of the biobed depth from 0.5 *m* to 1.5 *m*, reduced leaching of mobile and moderately mobile pesticides[74]. In biobeds receiving the highest water load, leaching was reduced with 77% and for biobeds receiving low water loading with 99% with increasing depth up to 1.5 *m.*

Table 1.3: Pesticide mass balances for a 0.5 m long biobed columns subjected to a hydraulic loading of 9747 L m^{-2}, 2797 L m^{-2} and 486 L m^{-2} [75].

	9747 L m^{-2}			2797 L m^{-2}			486 L m^{-2}		
	% leached	% degraded	% retained	% leached	% degraded	% retained	% leached	% degraded	% retained
isoproturon	6.37	93.53	0.10	0.20	99.71	0.09	0.000	98.81	1.19
pendimethalin	0.12	87.08	12.80	0.01	85.06	14.93	0.002	82.80	17.20
chlorpyriphos	0.04	99.52	0.44	0.01	99.27	0.71	0.002	96.51	3.49
chlorothalonil	0.11	98.04	1.85	0.01	98.30	1.70	0.001	90.55	9.45
epoxiconazole	0.05	66.41	33.54	0.00	66.08	33.92	0.001	67.15	32.85
dimethoate	6.08	93.90	0.02	0.61	99.37	0.02	0.004	99.88	0.11

1.6.4 Repeated use of pesticides

Repeated use of certain compounds over a number of seasons can result in enhanced degradation. This is probably due to adaptation and proliferation of specific microbial communities which utilise the compound as an energy source and thus degrade it more easily[76,77]. Repeated application of metalaxyl in a biomix constituted by topsoil, vine-branches and urban-waste-garden compost resulted in a sharp removal of the half-life[67]. INRA also observed that repeated treatments with certain pesticides increased the degradation potential of the microflora in a Phytobac© system[64]. Therefore it was suggested to farmers to fill their Phytobac© with adapted or pesticide-primed soil (i.e. soil originating from an agricultural field or other source which has been long-term treated with and exposed to the target pesticide and which has developed a pesticide-degrading micro population able to mineralize the compound from their own fields).

However Fogg *et al.*[48] found that the rate of pesticide degradation decreased with each additional application for isoproturon, cholorothalonil, chlorpyriphos, dimethoate and pendimethalin (Annex – parameter: repeated application). This might be caused by the absence of responsive microorganisms or essential co-actors, by unsuitable environmental conditions or by the presence of inhibitory factors[78]. It should be mentioned that the experiment was performed with high pesticide concentrations, therefore, it is likely that the negative effects of these high concentrations masked any increase in the microbial activity. Another plausible explanation is the existence of two differential microbial systems, a co-metabolic, mainly fungal, system occurring in the biobed that is not enhanced by repeated applications of pesticides and a metabolic system, mainly bacterial, occurring in the Phytobac©, the rate of which increases with enrichment of degrading microorganisms[61].

1.7 Conclusion

Contamination of ground and surface water puts pressure on the use of pesticides. Pesticide contamination of water can often be linked to point sources rather than to diffuse sources. Examples of such point sources are areas on farms where pesticides are handled, filled into sprayers and where sprayers are cleaned. To reduce contamination from these point sources, different kinds of biopurification systems are being researched in various member states of the EU. Biopurification is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. The systems available for bio-cleaning of pesticides vary according to their shape and design. Up till now three systems have been extensively described and reported: the biobed, the Phytobac© and the biofilter. Most of these constructions are excavations or different sizes of containers, filled with biological material. The function of this organic matrix is to retain and degrade pesticides applied to the system. Overall, from the available data, it could be concluded that for most of the pesticides, more than 95% was degraded after one year. Therefore it is strongly advisable to implement this system on-farm to reduce groundwater or surface water contamination.

ANNEX

Overview of the pesticides studied in biopurification experiments under variable conditions, such as different water loadings, different soil types, different concentrations, a mixture of pesticides, influence of repeated applications, different substratums, influence of biobed depth, influence of inoculation of the substratum and differing age of the system

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter(s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
atrazine	herbicide					52.1						White rot fungi	*Coriolus versicolor*	[79]
atrazine	herbicide					61.3						White rot fungi	*Hypholoma fasciculare*	[79]
atrazine	herbicide					57.3						White rot fungi	*Stereum hirsutum*	[79]
azoxystrobine	fungicide	50	$<6 \times 10^{-7}$									Leaching	After 312 days	[80]
bentazone	herbicide	50		11		20		80				Leaching	/	[58]
bromoxynil	herbicide	50		< 2				> 98				Leaching	/	[58]
chlorothalonil	fungicide	3060		0.11					99.89	98.04	1.85	Influence of water loading	9747 *L water* m^{-2}	[74]
chlorothalonil	fungicide	3060		0.01					99.98	98.3	1.7	Influence of water loading	2797 *L water* m^{-2}	[74]
chlorothalonil	fungicide	3060		0.001					99.99	90.55	9.45	Influence of water loading	486 *L water* m^{-2}	[74]
chlorothalonil	fungicide	445					90					Influence of soil	silty clay	[46]
chlorothalonil	fungicide	824				90						Influence of soil	sandy loam	[46]
chlorothalonil	fungicide	1699				90						Influence of soil	clay	[46]
chlorothalonil	fungicide	3118			90							Influence of concentration	7 $mg\ kg^{-1}$	[66]
chlorothalonil	fungicide	3118			90							Influence of concentration	14 $mg\ kg^{-1}$	[66]
chlorothalonil	fungicide	3118			90							Influence of concentration	29 $mg\ kg^{-1}$	[66]
chlorothalonil	fungicide	3118			90							Influence of concentration	57 $mg\ kg^{-1}$	[66]
chlorothalonil	fungicide	3118			50		90					Influence of concentration	143 $mg\ kg^{-1}$	[66]
chlorothalonil	fungicide	3118			50		90					Influence of concentration	287 $mg\ kg^{-1}$	[66]
chlorothalonil	fungicide	555			90							Influence of a mixture	only chlorothalonil	[66]
chlorothalonil	fungicide	555			90							Influence of a mixture	chlorothalonil+ isoproturon	[66]
chlorothalonil	fungicide	986.5			50	90						Influence of a mixture	single pesticide	[48]
chlorothalonil	fungicide	986.5			50	90						Influence of a mixture	mix of pesticides	[48]
chlorothalonil	fungicide	526.8			90							Repeated application	1st application	[48]
chlorothalonil	fungicide				90							Repeated application	2nd application	[48]
chlorothalonil	fungicide				50	90						Repeated application	3rd application	[48]
chlorothalonil	fungicide	96.8	< 0.0001									Leaching	after 10 days	[55]

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter(s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
chlorothalonil	fungicide	96.8	< 0.0001									Leaching	after 10 days	[55]
chlorpyriphos	insecticide					64						Matrix	Swedish biomix	[81]
chlorpyriphos	insecticide					64						Matrix	Swedish biomix	[81]
chlorpyriphos	insecticide					54						Matrix	Italian biomix	[81]
chlorpyriphos	insecticide					70%						Influence of concentration	10 $mg\ kg^{-1}$	[67]
chlorpyriphos	insecticide					54%						Influence of concentration	50 $mg\ kg^{-1}$	[67]
chlorpyriphos	insecticide	1468		0.04					99.96	99.52	0.44	Influence of water loading	9747 $L\ water\ m^{-2}$	[74]
chlorpyriphos	insecticide	1468		0.01					99.98	99.27	0.71	Influence of water loading	2797 $L\ water\ m^{-2}$	[74]
chlorpyriphos	insecticide	1468		0.002					99.99	96.51	3.49	Influence of water loading	486 $L\ water\ m^{-2}$	[74]
chlorpyriphos	insecticide	354.9				50		90				Influence of a mixture	single pesticide	[48]
chlorpyriphos	insecticide	354.9						50	90			Influence of a mixture	mix of pesticides	[48]
chlorpyriphos	insecticide	252.8				50		90				Influence of a mixture	1st application	[48]
chlorpyriphos	insecticide					50			90			Influence of a mixture	2nd application	[48]
chlorpyriphos	insecticide								50			Repeated application	3rd application	[48]
chlorpyriphos	insecticide	77.646	0.0004									Leaching	after 10 days	[55]
chorpyriphos	insecticide	750			100	100						Influence of substrate	substrate: citrus peel + green waste compost	[49]
chlorpyriphos	insecticide	750			100	100						Influence of substrate	substrate: citrus peel + urban waste compost	[49]
chlorpyriphos	insecticide	750			100	100						Influence of substrate	substrate: vine branch + green waste compost	[49]
chlorpyriphos	insecticide	750			100	100						Influence of substrate	substrate: vine branch + urban waste compost	[49]
chlorpyriphos	insecticide						65.49					Level of straw	50% straw	[42]
chlorpyriphos	insecticide						78.47					Level of straw	25% straw	[42]
chlorpyriphos	insecticide						68.74					Level of straw	12.5% straw	[42]
chlorpyriphos	insecticide						93.2					White rot fungi	*Coriolus versicolor*	[79]
chlorpyriphos	insecticide						29.0					White rot fungi	*Hypholoma fasciculare*	[79]

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter (s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
chlorpyriphos	insecticide						6.1					White rot fungi	*Stereum hirsutum*	[79]
cymoxanil	fungicide			0						100		Mass balance	Over 2 years	[82]
cyprodinil	fungicide			0						100		Mass balance	Over 2 years	[82]
dimethoate	insecticide	50	$<3 \times 10^{-7}$									Leaching	After 312 days	[80]
dimethoate	insecticide	694		6.08					93.92	93.9	0.02	Influence of water loading	9747 *L water* m^{-2}	[74]
dimethoate	insecticide	694		0.61					99.39	99.37	0.02	Influence of water loading	2797 *L water* m^{-2}	[74]
dimethoate	insecticide	694		0.004					99.99	99.88	0.11	Influence of water loading	486 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		1.41					98.59	98.52	0.07	Influence of depth and water loading	0.5m deep, 9747 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0.04					99.96	99.43	0.53	Influence of depth and water loading	1m deep, 9747 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0.32					99.68	99.6	0.08	Influence of depth and water loading	1.5m deep, 9747 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0.1					99.9	99.66	0.24	Influence of depth and water loading	0.5m deep, 2797 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0.12					99.88	99.67	0.21	Influence of depth and water loading	1m deep, 2797 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0.06					99.94	99.74	0.2	Influence of depth and water loading	1.5m deep, 2797 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0					100	99.94	0.06	Influence of depth and water loading	0.5m deep, 486 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0.0007					100	99.87	0.13	Influence of depth and water loading	1m deep, 486 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		0.0001					100	99.7	0.3	Influence of depth and water loading	1.5m deep, 486 *L water* m^{-2}	[74]
dimethoate	insecticide	435.2		1.36					98.64	98.64	0	Influence of soil	sandy loam	[46]
dimethoate	insecticide	435.2		1.64					98.36	98.36	0	Influence of soil	clay	[46]
dimethoate	insecticide	435.2		0.04					99.96	99.96	0	Influence of soil	silty clay	[46]
dimethoate	insecticide	167.5			90							Influence of a mixture	single pesticide	[48]
dimethoate	insecticide	167.5			50	90						Influence of a mixture	mix of pesticides	[48]

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter (s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
dimethoate	insecticide	119.4			50	90						Repeated application	1st application	48
dimethoate	insecticide				50		90					Repeated application	2nd application	48
dimethoate	insecticide					50		90				Repeated application	3rd application	48
diuron	herbicide					52.3						White rot fungi	*Coriolus versicolor*	79
diuron	herbicide					15.9						White rot fungi	*Hypholoma fasciculare*	79
diuron	herbicide					74.1						White rot fungi	*Stereum hirsutum*	79
diuron	herbicide	50	$< 2 \times 10^{-7}$									Leaching	After 312 days	80
fenitrothion	insecticide			0						58.5		Mass balance	Over 2 years	82
fenpropimorph	fungicide	50	$< 1 \times 10^{-6}$									Leaching	After 312 days	80
fluazifop	herbicide	50	6.8×10^{-6}									Leaching	After 312 days	80
fludioxonil	fungicide			0						100		Mass balance	Over 2 years	82
flufenoxuron	fungicide			0						92.6		Mass balance	Over 2 years	82
glyphosate	herbicide	50	0									Leaching	After 312 days	80
ioxynil	herbicide	50	$< 5 \times 10^{-7}$									Leaching	After 312 days	80
iprodione	fungicide					57.9						White rot fungi	*Coriolus versicolor*	79
iprodione	fungicide					47.4						White rot fungi	*Hypholoma fasciculare*	79
iprodione	fungicide					62.3						White rot fungi	*Stereum hirsutum*	79
iprovalicarb	fungicide			2.5						25.5		Mass balance	Over 2 years	82
isoproturon	herbicide	3200		0.002					100	99.71	0.29	Influence of depth and water loading	0.5m deep, 9747 *L water* m^{-2}	74
isoproturon	herbicide	3200		0.0003					100	99.93	0.07	Influence of depth and water loading	1m deep, 9747 *L water* m^{-2}	74
isoproturon	herbicide	3200		0.0001					100	99.56	0.44	Influence of depth and water loading	1.5m deep, 9747 *L water* m^{-2}	74
isoproturon	herbicide	3200		0.0001					100	98.94	1.06	Influence of depth and water loading	0.5m deep, 2797 *L water* m^{-2}	74
isoproturon	herbicide	1233			<95							Influence of depth and water loading	1m deep, 2797 *L water* m^{-2}	74

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter (s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
isoproturon	herbicide	2543			< 95							Influence of depth and water loading	1.5m deep, 2797 *L water* m^{-2}	74
isoproturon	herbicide	665				90						Influence of depth and water loading	0.5m deep, 486 *L water* m^{-2}	74
isoproturon	herbicide	3200		0.006					100	99.5	0.5	Influence of depth and water loading	1m deep, 486 *L water* m^{-2}	74
isoproturon	herbicide	3200		0.007					99.99	99.61	0.38	Influence of depth and water loading	1.5m deep, 486 *L water* m^{-2}	74
isoproturon	herbicide	3200		0.002					100	99.49	0.51	Influence of soil	silty clay	46
isoproturon	herbicide	1233				90						Influence of soil	sandy loam	46
isoproturon	herbicide	2543				90						Influence of soil	clay	46
isoproturon	herbicide	665						90				Influence of soil	silty clay	46
isoproturon	herbicide	5190			50	90						Influence of concentration	11 $mg\ kg^{-1}$	66
isoproturon	herbicide	5190			50	90						Influence of concentration	23 $mg\ kg^{-1}$	66
isoproturon	herbicide	5190			50	90						Influence of concentration	46 $mg\ kg^{-1}$	66
isoproturon	herbicide	5190			50	90						Influence of concentration	91 $mg\ kg^{-1}$	66
isoproturon	herbicide	5190				50	90					Influence of concentration	228 $mg\ kg^{-1}$	66
isoproturon	herbicide	5190				50	90					Influence of concentration	456 $mg\ kg^{-1}$	66
isoproturon	herbicide	925			50	90						Influence of a mixture	only isoproturon	66
isoproturon	herbicide	925			50	90						Influence of a mixture	isoproturon + chlorothalonil	66
isoproturon	herbicide	1233			50	90						Influence of a mixture	single pesticide	48
isoproturon	herbicide	1233				50	90					Influence of a mixture	mix of pesticides	48
isoproturon	herbicide	874.3			50	90						Repeated application	1st application	48
isoproturon	herbicide					50		90				Repeated application	2nd application	48
isoproturon	herbicide							90				Repeated application	3rd application	48
isoproturon	herbicide	140.850	< 0.0005									Leaching	after 10 days	55

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter(s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
isoproturon	herbicide	100				58	76					Influence of inoculation	without inoculation of microorganisms	20
isoproturon	herbicide	100				93	>99					Influence of inoculation	with inoculation of microorganisms	20
kresoxim-methyl	fungicide	50	$< 5 \times 10^{-7}$									Leaching	After 312 days	80
linuron	herbicide	50	$< 6 \times 10^{-7}$									Leaching	After 312 days	80
mancozeb	fungicide			2						98		Mass balance	Over 2 years	82
MCPA	herbicide	50	$< 5 \times 10^{-7}$									Leaching	After 312 days	80
mecoprop	herbicide	2000	2090	12.9								Influence of age of biobed	1st year	48
mecoprop	herbicide	2000	0	0								Influence of age of biobed	2nd year	48
mecoprop	herbicide	1536		2.07					97.93	97.93	0	Influence of depth and water loading	0.5m deep, 9747 *L water* m^{-2}	74
mecoprop	herbicide	1536		1.02					98.98	98.98	0	Influence of depth and water loading	1m deep, 9747 *L water* m^{-2}	74
mecoprop	herbicide	1536		3.37					96.63	96.63	0	Influence of depth and water loading	1.5m deep, 9747 *L water* m^{-2}	74
mecoprop	herbicide	1536		1.54					98.46	98.46	0	Influence of depth and water loading	0.5m deep, 2797 *L water* m^{-2}	74
mecoprop	herbicide	1536		3.37					96.63	96.63	0	Influence of depth and water loading	1.5m deep, 9747 *L water* m^{-2}	74
mecoprop	herbicide	1536		1.54					98.46	98.46	0	Influence of depth and water loading	0.5m deep, 2797 *L water* m^{-2}	74
mecoprop	herbicide	1536		0.33					99.67	99.67	0	Influence of depth and water loading	1m deep, 2797 *L water* m^{-2}	74
mecoprop	herbicide	1536		0.11					99.89	99.89	0	Influence of depth and water loading	1.5m deep, 2797 *L water* m^{-2}	74
mecoprop	herbicide	1536		0.0005					100	100	0	Influence of depth and water loading	0.5m deep, 486 *L water* m^{-2}	74
mecoprop	herbicide	1536		0.0006					100	100	0	Influence of depth and water loading	1m deep, 486 *L water* m^{-2}	74

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter(s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
mecoprop	herbicide	1536		0.0009					100	100	0	Influence of depth and water loading	1.5m deep, 486 *L water* m^{-2}	74
mecoprop	herbicide	571			90							Influence of soil	sandy loam	46
mecoprop	herbicide	1177			90							Influence of soil	clay	46
mecoprop	herbicide	308			90							Influence of soil	silty clay	46
mecoprop	herbicide	1536		0.02					99.98	99.50	0.48	Influence of soil	sandy loam	46
mecoprop	herbicide	1536		0.112					99.89	99.89	0	Influence of soil	clay	46
mecoprop	herbicide	1536		0.004					99.99	99.38	0.61	Influence of soil	silty clay	46
mecoprop	herbicide	571			90							Influence of soil	sandy loam	46
mecoprop	herbicide	1177			90							Influence of soil	clay	46
mecoprop	herbicide	308			90							Influence of soil	silty clay	46
mecoprop	herbicide	50		< 2%			> 98					leaching	/	58
metalaxyl	fungicide			1						97		Mass balance	Over 2 years	82
metalaxyl	fungicide					39.9						White rot fungi	*Coriolus versicolor*	79
metalaxyl	fungicide					53.9						White rot fungi	*Stereum hirsutum*	79
metalaxyl	fungicide	960			97	100						Influence of substrate	substrate: citrus peel + green waste compost	49
metalaxyl	fungicide	960			89	97						Influence of substrate	substrate: citrus peel + urban waste compost	49
metalaxyl	fungicide	960			14	37						Influence of substrate	substrate: vine branch + green waste compost	49
metalaxyl	fungicide	960			0	100						Influence of substrate	substrate: vine branch + urban waste compost	49
metalaxyl	fungicide				27							Repeated application	1^{st} application	67
metalaxyl	fungicide				71							Repeated application	2^{nd} application	67
metalaxyl	fungicide				100							Repeated application	3^{rd} application	67
metamitron	herbicide	50	$< 7 \times 10^{-7}$									leaching	After 312 days	80
methabenzthiazuron	herbicide	50				31		53				leaching	/	58

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter(s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
metribuzin	herbicide	0	4.7×10^{-6}									Leaching	After 312 days	[80]
metribuzin	herbicide	50		< 2%		66		96				leaching	/	[58]
metsulfuron-methyl	herbicide	7.68		100					0	0	0	Influence of depth and water loading	0.5m deep, 9747 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		19.34					80.66	80.66	0	Influence of depth and water loading	1m deep, 9747 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		15.29					84.71	84.71	0	Influence of depth and water loading	1.5m deep, 9747 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		48.34					51.66	51.66	0	Influence of depth and water loading	0.5m deep, 2797 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		18.38					81.62	81.62	0	Influence of depth and water loading	1m deep, 2797 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		5.94					94.06	94.06	0	Influence of depth and water loading	1.5m deep, 2797 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		0.24					99.76	99.76	0	Influence of depth and water loading	0.5m deep, 486 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		0.0003					100	100	0	Influence of depth and water loading	1m deep, 486 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	7.68		0.0002					100	100	0	Influence of depth and water loading	1.5m deep, 486 *L water* m^{-2}	[74]
metsulfuron-methyl	herbicide	34				90						Influence of soil	sandy loam	[46]
metsulfuron-methyl	herbicide	71					90					Influence of soil	clay	[46]
metsulfuron-methyl	herbicide	18						90				Influence of soil	silty clay	[46]

Pesticide	Type	Input ($mg\ L^{-1}$)	Rest in leachate		Sum of degradation and retention (%)					Degra-dation (%)	Reten-tion (%)	Studied parameter(s)	Specifications	Ref.
			$mg\ L^{-1}$	%	<20*d*	<60*d*	<100*d*	<180*d*	<365*d*					
metsulfuron-methyl	herbicide	34						90				Influence of soil	sandy loam	[46]
metsulfuron-methyl	herbicide	71					90					Influence of soil	Clay	[46]
metsulfuron-methyl	herbicide	18					90					Influence of soil	silty clay	[46]
penconazole	fungicide			0						100		Mass balance	Over 2 years	[82]
pendimethalin	herbicide	4080		0.12					99.88	87.08	12.8	Influence of water loading	9747 *L water* m^{-2}	[74]
pendimethalin	herbicide	4080		0.01					99.99	85.06	14.93	Influence of water loading	2797 *L water* m^{-2}	[74]
pendimethalin	herbicide	4080		0.002					99.99	82.8	17.2	Influence of water loading	486 *L water* m^{-2}	[74]
pendimethalin	herbicide	739.8				50		90				Influence of a mixture	single pesticide	[48]
pendimethalin	herbicide	739.8					50		90			Influence of a mixture	mix of pesticides	[48]
pendimethalin	herbicide	702.5				50	90					Repeated application	1st application	[48]
pendimethalin	herbicide					50			90			Repeated application	2nd application	[48]
pendimethalin	herbicide							50				Repeated application	3rd application	[48]
pendimethalin	herbicide	196.790	0									Leaching	after 10 days	[57]
pirimicarb	insecticide	50		< 2%		20		80				Leaching	/	[58]
propiconazole	fungicide	50				22		53				Leaching	/	[58]
propyzamide	herbicide	50	$<6 \times 10^{-7}$									Leaching	After 312 days	[80]
prosulfocarb	herbicide	50	$< 1 \times 10^{-6}$									Leaching	After 312 days	[80]
terbuthylazine	herbicide					39.9						White rot fungi	*Coriolus versicolor*	[79]
terbuthylazine	herbicide					37.0						White rot fungi	*Hypholoma fasciculare*	[79]
terbuthylazine	herbicide					78.6						White rot fungi	*Stereum hirsutum*	[79]
terbuthylazine	herbicide	50	$< 3 \times 10^{-7}$									Leaching	After 312 days	[80]

Chapter 2: Research objectives, thesis outline, and pesticide, substratum selection

In general, studies performed on the use of on-farm biopurification systems to treat pesticide contaminated water mostly focused on mass balances *i.e.* the input concentration was compared with the outlet concentration to determine the efficiency of the biopurification system. An overview of these studies was provided in Chapter 1. However, so far, the system worked as a black box without really characterizing the processes occurring inside the system and the main parameters influencing its efficiency. Therefore some unanswered questions still remained. The quality of the substratum was questioned, how long will substratums be active and able to degrade pesticides, and which is the ideal composition of the substratums? What are the optimal dimensions for a certain hydraulic load generated on-farm? Which pesticides need to be treated with extra care? Which processes (*e.g.* retention and/or degradation) occur inside the system? How do the residues inside the matrix evolve after use? Therefore, more profound studies on these topics were necessary. From such studies, recommendations can follow to provide guidelines on the implementation of the biopurification system, on the ideal substratum composition, on the use of inoculation sources, etc.

2.1 Research objectives and thesis outline

The specific objectives of this PhD thesis are:

(1) To characterize sorption kinetics and isotherms of pesticides on a range of organic substratums

(2) To determine pesticide retention and degradation of pesticides in micro and macro scale column experiments

(3) To assess the influence of inoculation with pesticide-primed material on transport and degradation of pesticides

(4) To study the transport and fate of pesticides in micro- and macrocosms subjected to a variable water flux

(5) To verify the possibility of large and small scale composting/incubation on the removal of pesticide residues in the organic matrix

This research is performed at an increasing scale (Figure 2.1). Firstly, retention and degradation experiments are carried out on a small scale (in batch) to better understand the ongoing processes. To validate the obtained results in batch, small scale column experiments were performed, followed by an up scaling to large scale columns. The outline of this thesis is based on this up scaling and the different chapters will shortly be introduced below.

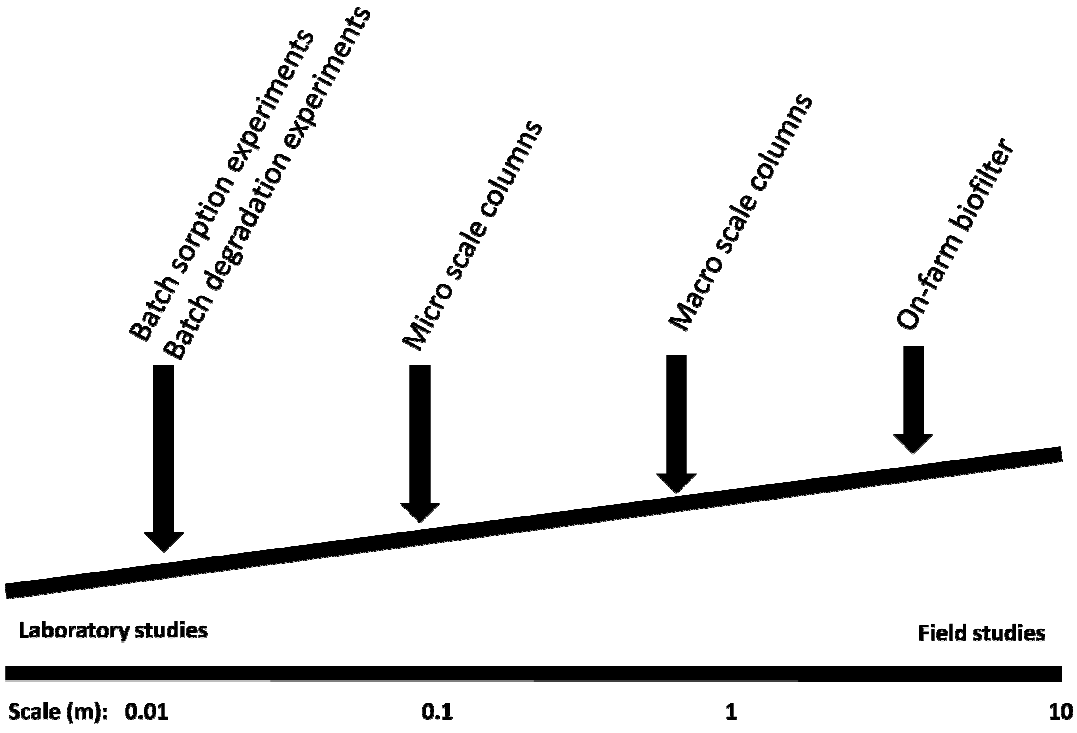


Figure 2.1: Spatial scale of experimental studies

A first section (Chapter 3) focuses on the sorptive behavior of pesticides on the matrix substratums used in biopurification systems. As the efficiency of the biopurification system depends on retention and degradation of pesticides, it is important to get a perspective on the behavior of pesticides with varying physico-chemical characteristics on biopurification substratums with different organic matter content. This in order to determine the main factors which determine the sorption process and to be able to increase the efficiency of the system by implementing pesticide-substratum combinations with a sufficient retention. Consequently batch sorption experiments were carried out to assess the sorption kinetics and sorption isotherms. Sorption kinetics are essential in describing the non-equilibrium phase of pesticide sorption on organic substratums, while sorption isotherms provide parameters which are applicable in the modeling part discussed in the following section.

In the second section (Chapter 4) the aim was to validate the obtained results from chapter 3. Retention of pesticides can also be determined by performing column displacement experiments. By the use of a pesticide step input a breakthrough curve was generated. Through inverse modeling, retention and degradation parameters could be determined. A comparison with the previously obtained results was carried out. Furthermore, the influence of different combinations of organic substratums on pesticide retention was assessed on a small and large scale. Batch degradation experiments were also performed, with material originating from the column studies to study the degradation potential of the pesticide degrading community in the micro- and macrocosms.

The third section (Chapter 5) focuses on degradation of pesticides when columns are inoculated with so-called pesticide-primed material previously treated with the target pesticides. Fate of pesticides in the inoculated and non-inoculated matrix was assessed in small scale columns. All treatments were compared with a column with a non-primed reference soil to test the advantage of the inoculation of adapted or pesticide primed soil.

The fourth section (Chapter 6) studies the effect of various hydraulic loads on the transport and fate of pesticides in micro- and macrocosms in order to determine the optimal dimensions of the biopurification system or the maximum hydraulic load that a biopurification system can process. Three different flows were applied to small and large scale columns. Transport and degradation of the applied pesticides were determined by inverse modeling and batch degradation experiments.

The fifth section (Chapter 7) focuses on the disposal of a used biomix. Heaping the biomix above ground on an impermeable surface may result in mobile pesticides leaching from the biomix and this could end up in possible contamination of adjacent surface waters. Therefore, to reduce the residues present in the matrix, two composting processes were tested. A small scale composting was carried out in cooperation with PCFruit in small compost barrels, while the large scale composting was performed in an industrial composting process.

The sixth section (Chapter 8) provides a brief recapitulation of the main findings and discusses the practical implications and future prospects of the research conducted.

2.2 Pesticide selection

The use of pesticides is quite complex. Depending on the crop and season different pesticides are used. The characteristics of the pesticides used are very diverse: from poor soluble to very soluble, from harmless to very toxic, from highly degradable to very persistent, from very mobile to immobile in the soil. Studying all products is too expensive and time consuming. Therefore to reduce the amount of tests, pesticides were grouped based on their physicochemical characteristics and biodegradability. The classification also takes into account pesticides which have been frequently detected in surface and ground water in Flanders and which are frequently used. Pesticides were divided into four categories based on their persistence ($t_{1/2}$ value (d)) and mobility (K_{oc} ($L\ kg^{-1}$)), two parameters governing leaching of pesticides. The $t_{1/2}$ value is the time needed for 50% decrease of the initial pesticide concentration, and the K_{oc} value is the organic carbon partition coefficient. Pesticides were classified as persistent when the $t_{1/2}$ value was higher than 90 days[83] and as immobile when the log K_{oc} value was higher than 2.5. This value is based on the GUS (Groundwater Ubiquity Score) indicator[84]:

$$GUS = \log t_{1/2} \cdot (4 - \log K_{oc}) \qquad (2.1)$$

A GUS indicator lower than 1.8 indicates an immobile pesticide[8]. This leads to the classification of pesticides into four categories: persistent-immobile, persistent-mobile, non-persistent-mobile and non-persistent-immobile. One or more pesticides were selected out of each category to be used in batch sorption experiments in Chapter 3 (Figure 2.2).

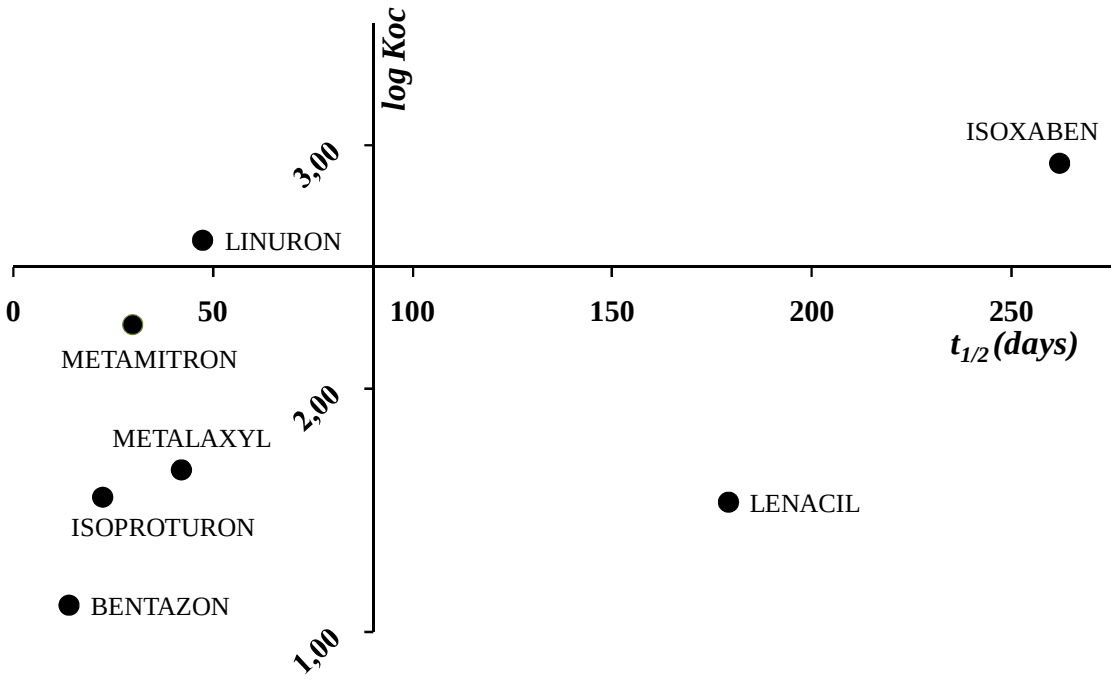


Figure 2.2: Selected pesticides presented as a function of persistence ($t_{1/2}$) and mobility (log K_{oc})

Pesticides selected out of the non-persistent-mobile category were metalaxyl (methyl N-(2,6-dimethylphenyl)-N-(methoxyacethyl)-DL-alaninate) (K_{oc}= 47 *L* kg^{-1}, $t_{1/2,soil}$ = 42 *d*), metamitron (3-Methyl-4-amino-6-phenyl-1,2,4-triazin-5(4H)-one) (K_{oc}= 185 *L* kg^{-1}, $t_{1/2,soil}$ = 30 *d*) and isoproturon (*N,N*-dimethyl-*N*'-[4-(1-methylethyl)phenyl]urea) (K_{oc}= 36 *L* kg^{-1}, $t_{1/2,soil}$ = 22,5 *d*). Linuron (3-3,4 dichlorophenyl-1-methoxy-1-methyl-urea) was selected as a non-persistent-immobile pesticide (K_{oc}= 410 *L* kg^{-1}, $t_{1/2,soil}$ = 47,5 *d*). Lenacil (3-cyclohexyl-6,7-dihydro-1*H*-cyclopentapyrimidine-2,4(3*H,*5*H*)-dione) ((K_{oc}= 34 *L* kg^{-1}, $t_{1/2,soil}$ = 179 *d*) was selected to represent the persistent-mobile category. Isoxaben was chosen as a persistent, immobile pesticide (K_{oc}= 862, $t_{1/2,soil}$ = 262 *d*)[85]. Their structural formulas are presented in Figure 2.3.

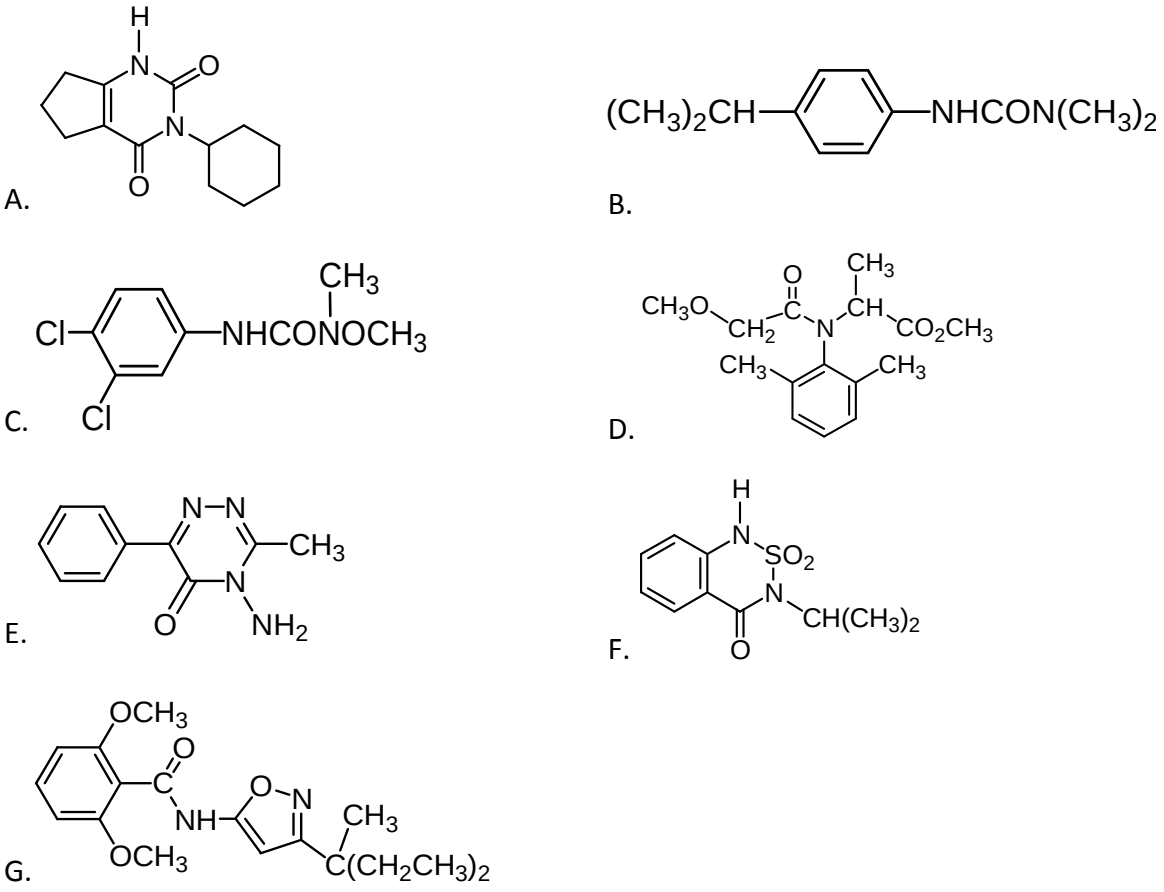


Figure 2.3: Structural formulas of pesticides used in this study A. lenacil, B. isoproturon, C. linuron, D. metalaxyl, E. metamitron, F. bentazon, G. isoxaben.

2.3 Substratum selection

Selected substratums were substratums commonly used in biopurification systems, *i.e.* peat mix, garden waste compost, straw, sandy loam soil, cow manure, coconut chips and willow choppings. Peat mix, garden waste compost and straw serve as an important nutrient source for pesticide degrading microorganisms due to their high organic matter content. Peat consists of a mixture of black and white peat and was chosen because this material has been frequently used in other studies on biopurification systems which facilitates comparison. Concerning compost, the preference was given to garden waste compost as this mineralizes slower and is less heterogenic compared to compost originating from vegetable, fruit and garden waste. Straw, which can serve as a nutrient source for white-rot fungi, has been used in the majority of the studies and is easily available and cheap. The sandy loam soil, which is a common soil type in Belgium, acts as an inoculum of pesticide degrading microorganisms for the biopurification system[16]. This soil did not have any history of pesticide use and was not expected to contain any intrinsic pesticide degrading capacity. Cow manure, which is rich in nutrients, improved the efficacy of the system in the study of Genot *et al.*[50]. Finally, coconut chips and willow chopping have been chosen as structure elements and as slow-degrading carbon sources. Coconut chips have the ability to buffer a high quantity of water and can therefore prevent saturation of the system. This is not native material but is reasonably cheap. Willow chopping was selected due to its local availability. These substratums also provide lignin for lignin degrading microorganisms, which produce enzymes catalyzing the degradation of a broad spectrum of chemicals[41]. The main physicochemical characteristics of the studied substratums are presented in Table 2.1. All substratums were air-dried. Coco chips consist of the outer shell of a coco nut and is cut to smaller pieces +/- 1 x 1 *cm* before use. Straw and willow chopping were chopped to fragments of 2-3 *cm*. The sandy loam soil (33% sand, 56% loam and 11% clay) was crushed to pass a 2 *mm* sieve. Cow manure (DCM, The Netherlands) was added in the form of dried pellets.

Table 2.1: Physicochemical characteristics of the substratums used

	pH	***OC* ($g\ kg^{-1}$)**	***P_2O_5* ($g\ kg^{-1}$)**	***K_2O* ($g\ kg^{-1}$)**	***CaO* ($g\ kg^{-1}$)**	***MgO* ($g\ kg^{-1}$)**	***Na* ($mg\ 100\ g^{-1}$)**	***CEC* ($meq\ 100\ g^{-1}$)**	***Specific density* ($mg\ m^{-3}$)**
Garden waste compost	7.7	278.4	3.1	6.2	15.7	4.0	ND*	ND	1.84
Willow chopping	5	427.6	9.3	36.4	29.4	7.8	0.26	28.44	1.57
Straw	6.6	423.6	6.9	77.2	3.8	3.6	0.57	25.21	1.56
Coco chips	6.7	446.9	9.8	123.9	0.8	1.0	2.52	58.98	1.67
Peat mix	6.4	476.1	4.5	8.0	670.0	7.9	0.42	129.42	1.58
Sandy loam soil	6.9	9.1	0.3	4.2	1.9	0.8	0.21	13.87	2.81
Cow manure	6.9	375.9	98.7	113.7	18.0	49.2	1.41	53.22	1.68

*ND= not determined

Chapter 3: Sorption characteristics of pesticides on matrix substratums used in biopurification systems

This chapter has been compiled from:

De Wilde, T., Mertens, J., Spanoghe, P., Ryckeboer, J., Jaeken, P. , Springael, D. 2008. Sorption kinetics and its effects on retention and leaching. Chemosphere, 72, 509-516.

De Wilde, T., Spanoghe, P., Ryckeboer, J., Springael, D., Jaeken, P. 2009. Sorption characteristics of pesticides on matrix substrates used in biopurification systems. Chemosphere, 75, 100-108.

3.1 Abstract

Sorption of pesticides to substratums used in biopurification systems is important as it controls in a major way the system's efficiency. Ideally, pesticide sorption should occur fast and at a high efficiency so that leaching of the pesticide in the biopurification system is minimized. In this chapter, batch sorption kinetics and sorption isotherms for various pesticides were determined on substratums commonly used in a biopurification system. Sorption appeared to be fast for immobile pesticides. Simulations performed with the obtained first- order kinetic constant showed that slow sorption can significantly influence the leaching potential of mobile pesticides. At equilibrium, the Freundlich isotherm was found to be the most suitable model to fit the experimental data. More immobile pesticides tended to associate stronger with the organic substratum. According to increasing sorption capacity, the substratums could be classified as peat mix > compost, coco chips, straw > cow manure, willow chopping > sandy loam soil. Furthermore, it was observed that sorption of a formulated pesticide was not significantly different from the sorption of the active substance. Secondly, competitive sorption was observed for metalaxyl and linuron. Finally, the individual sorption coefficient K_d was additive, which means that sorption coefficients associated with individual substratums can be used to calculate the sorption coefficients associated with a mixture of substratums.

3.2 Introduction

Several techniques have been developed for the removal of pesticides from water. Sorption on activated carbon is the most widespread technology used to purify water contaminated by pesticides and other hazardous chemicals[86,87]. However, due to the high cost of activated carbon, its use in the field is sometimes restricted. Moreover, the high cost associated with its regeneration led to explore new inexpensive materials[88]. For the last decades, sorption of contaminants by sorbents of natural origin has gained important credibility due to the good performance and low cost of these complex materials[89-93]. This principle was also transferred to biopurification systems, which use natural materials (agricultural waste products or products available on-farm) to create a pesticide retaining and degrading environment to clean contaminated water on-farm.

The composition and type of organic material present in the biobed are believed to be crucial for retention of chemicals as well as for the amount and activity of microorganisms responsible for degradation of the pesticides[40]. Generally, the matrix in a biopurification system is composed of a structure material with a good porosity, retaining water and air (*e.g.* peat, green waste compost), an easily degradable organic material as a source of nutrients (*e.g.* straw, manure, compost) and an inoculation source of microorganisms such as topsoil. Matrix substratums that can be used in a biopurification system can have different organic carbon contents and since sorption of pesticides is mainly controlled by the organic matter content[94], sorption capacity of the substratums will be different.

Aiming at maximizing the organic carbon partition coefficient K_{oc} or sorption coefficient K_d to increase sorption, is not always sufficient. Knowledge of the sorption kinetics of the different pesticides to the different substratums used in a biopurification system may be important since it controls the sorption process efficiency. Ideally, pesticide sorption should occur fast ('equilibrium' sorption) so that leaching through the biopurification system is reduced and the time for microbial degradation increased. A number of studies have been published which show considerable variation in the time necessary for the establishment of equilibrium[95].

In many mathematical models describing leaching and sorption, the sorption process is often simplified by assuming that sorption attains instant equilibrium ('equilibrium' sorption). However, it has been reported that non-equilibrium processes likely occur during the transport of pesticides in soils[96]. Non-equilibrium is mostly caused by intrasorbent diffusion, which results from rate-limited mass transfer of sorbate from the exterior surface of the sorbent into the interior of the sorbent matrix[96].

Although sorption on different types of soil has been characterized for a wide variety of pesticides, information is missing on the sorption of pesticides on organic materials such as compost, peat and straw commonly used in on-farm biopurification systems. These data are essential to model the transport of pesticides in biopurification systems. The transport of pesticides in water in the system can be described with the convection dispersion equation. In order to accurately use this equation, it is necessary to determine as much parameters (retention and degradation parameters) as possible. This model could then be used to

predict the behavior of pesticides in the system and could identify problem pesticides which will be difficult to treat.

The overall objective of this study was to determine sorption kinetics and isotherms for pesticides on a wide range of organic materials likely to be present in a biopurification system. The sorption kinetics and sorption isotherms of a number of pesticides with different physicochemical characteristics on seven substratums (peat mix, garden waste compost, straw, sandy loam soil, cow manure, coconut chips and willow chopping) with variable composition were studied.

A mass transfer model was fitted to the observed pesticide concentrations in time resulting in an estimated kinetic rate constant α. This kinetic rate parameter allowed the identification of the substratums exhibiting fast sorption kinetics with respect to each of the five pesticides. Substratums which exhibit a rather slow sorption are considered to be more submissive to leaching and are therefore less suitable for use in a biopurification systems. To evaluate the effect of differences in α values on the breakthrough of pesticides through a biopurification system, the HYDRUS 1D model was used.

3.3 Materials and Methods

3.3.1 Chemicals

Metalaxyl, isoproturon, linuron, lenacil, bentazone and isoxaben of analytical standard grade (99%) were purchased from Riedel-de Haen, Seelze, Germany. Bentazone was not used in the study of the sorption kinetics. Technical grade metalaxyl (95.5 % purity) was kindly supplied by Syngenta (Basel, Switzerland). Isoproturon, linuron, isoxaben, bentazone and lenacil were respectively applied under the formulations Isoguard 83 WG (83% isoproturon, Gharda Chemicals Ltd., Surrey, England), Afalon SC (450 $g\ L^{-1}$ linuron, Makhteshim Agan Holland B.V., Ieper, Belgium), AZ 500 (500 $g\ L^{-1}$ isoxaben, Dow Agro Science, Wilrijk, Belgium), Basagran SG (87% bentazon, BASF, Antwerpen, Belgium) and Lenacil Protex 500 SC (500 $g\ L^{-1}$ lenacil, Protex, Deurne, Belgium). Methanol, acetonitrile, water were of A.R. grade (VWR, Leuven, Belgium).

Pesticides were dissolved in a 0.01 *M* solution of calcium chloride ($CaCl_2$) (Merck, Darmstadt, Germany) and 200 $mg\ L^{-1}$ sodium azide (NaN_3) (Sigma-Aldrich, St-Louis, MO, USA) in distilled water. $CaCl_2$ was used as a background electrolyte to simulate an ionic strength similar to that of a natural soil solution[97], while NaN_3 was added to minimize biological activity. To construct the sorption isotherms, aqueous pesticide solutions were prepared as concentrations of 1, 10, 1000, 2500 and 5000 $mg\ L^{-1}$. This range was based on the concentrations applied in the field. A high concentration could simulate a spill during filling, while low concentrations represented diluted waste water from cleaning the spraying equipment.

3.3.2 Sorption studies

Pesticide sorption on seven substratums was studied using the batch equilibrium technique according to the OECD guideline 106[98]. The sorption kinetic study was carried out in duplicate for two pesticide concentrations, *i.e.* 10 *mg* L^{-1} and 1000 *mg* L^{-1}, while sorption isotherms were studied in the concentration range described in paragraph 3.3.1. In tests with the sandy loam soil, garden waste compost, willow chopping and dried cow manure, 2.000 ± 0.001 *g* of air dried substratum was shaken in 250 *mL* conical flasks with 20 *mL* of 0.01 *M* $CaCl_2$ solution of the respective pesticide (1:10 solid liquid ratio). In tests with coconut chips, peat mix and straw, 2.000 *g* of air dried substratum was mixed with *50 mL* of 0.01 *M* $CaCl_2$ solution of the respective pesticide (1:25 solid liquid ratio). A higher volume of $CaCl_2$ solution was used for the latter substratums as these materials have a high water absorbing capacity. The mixtures were shaken in the dark on an orbital shaker at 150 *rpm* at room temperature. To study sorption kinetics, suspensions were filtered over an ash less paper filter (Whatman 589/1, pore size: 12-25 *µm*) after 0, 2, 7, 24, 48 and 72 *h* of shaking. Batch sorption experiments were performed to asses sorption of the studied pesticides on filter paper but no significant sorption was observed (data not shown). The residual pesticide concentration was determined in the filtrate. A control without added substratum was performed in order to correct for possible pesticide degradation during the shaking period.

Adsorbed pesticide concentration was calculated as:

$$C_s = \frac{V}{M_{sub}} \cdot \left(C_{l,ini} - C_l\right) \tag{3.1}$$

in which C_s (*mg* kg^{-1}) represents the adsorbed concentration, *V* (*L*), the volume of pesticide solution added, M_{sub} (*kg*) the weight of the substratum, $C_{l,ini}$ (*mg* L^{-1}) the initial pesticide concentration and C_l (*mg* L^{-1}) the concentration of pesticide in the liquid phase after incubation. After determining the equilibrium time, this time was used as the shaking time to obtain the sorption isotherms.

3.3.3 Pesticide concentration analysis

The filtrate was additionally filtrated with a syringe filter with a PVDF membrane with a pore size of 0.22 *µm* (Carl Roth, Karlsruhe-Rheinhafen, Germany). This aliquot was analyzed by HPLC-DAD UV analysis, performed on a Finnigan Surveyor HPLC (Thermo Electron Corporation; Waltham, MA, USA) equipped with a gradient pump, a degasser, an autosampler, and a diode array detector (DAD). The analytical column used was an Alltima HP C18 EPS 3 *µm* 150 *mm* x 3.0 *mm* (Alltech Associates Inc. Deerfield, IL, USA). The detector was set at a wavelength of 208 *nm*. The mobile phase consisted of an acetonitrile (solvent A) - 0.1*%* H_3PO_4 water (solvent B) solution at a linear gradient from 32*%* to 60*%* of solvent A in 4 *min* and in 4 *min* from 60*%* to 35*%* of solvent A, with 4 *min* for equilibrating the column. Flow rate was 0.7 *mL* min^{-1} and the volume injected was 10 *µL*.

Retention times of lenacil, bentazone, metalaxyl, isoproturon, linuron and isoxaben were respectively, 2.60 *min*, 3.00 *min*, 3.86 *min*, 4.40 *min*, 6.43 *min*, 8.58 *min*. Detection limits

(LOD) were 0.43 *µg L*$^{-1}$ for metalaxyl, 0.22 *µg L*$^{-1}$ isoproturon, 0.31 *µg L*$^{-1}$ for linuron, 0.49 *µg L*$^{-1}$ for lenacil, 0.28 *µg L*$^{-1}$ for bentazon and 0.58 *µg L*$^{-1}$ for isoxaben. The LODs were estimated from the injection of matrix-matched standard solutions with low concentration levels giving a signal-to-noise ratio of 3. All pesticides showed a linearity in the range of 0.1 to 10 *mg L*$^{-1}$ between HPLC output and coefficient of determination higher than 0.999. Recovery of the pesticides from the water was sufficiently high, with values of 95.62 (± 1.69)*%* for lenacil, 99.26 (± 2.54)*%* for bentazone, 94.03 (± 1.76)*%* for metalaxyl, 98.02 (± 1.21)*%* for isoproturon, 96.62 (± 2.18)*%* for linuron and 97.05 (± 3.12)*%* for isoxaben.

3.3.4 Data analysis

3.3.4.1 Sorption kinetics

A simple mass balance model combined with first order sorption kinetics was used to simulate the pesticide concentrations in time (*h*):

$$M_{tot} = M_s + M_l \tag{3.2}$$

where M_{tot} is the total amount of pesticide (*mg*), M_s is the amount of pesticide adsorbed on the solid phase (*mg*) and M_l is the amount of pesticide present in the liquid phase (*mg*). Equation (3.2) can be rewritten as:

$$C_{l,ini}V = C_s M_{sub} + C_l \cdot V \tag{3.3}$$

where $C_{l,ini}$ (*mg L*$^{-1}$) is the initial pesticide concentration added to the solid phase, V (*L*) is the volume of pesticide solution added to the solid phase, C_s (*mg kg*$^{-1}$) the sorbed pesticide concentration and M_{sub} (*kg*) the dry weight of the substratum used in the batch experiment.

Transfer of a pesticide to and from a solid is assumed to be driven by the difference between the amount currently adsorbed and the amount that would be adsorbed if the system was in equilibrium[99]:

$$\frac{dC_s}{dt} = \alpha \cdot (C_{s,eq} - C_s) \tag{3.4}$$

where α (h^{-1}) is a first-order kinetic constant and $C_{s,eq}$ (*mg kg*$^{-1}$) the sorbed concentration at equilibrium. This approach has been called a linear driving force (LDF) approximation[100] or a first-order mass transfer model[101]. The quantity $(C_{s,eq} - C_s)$ is a forcing function which is proportional to the difference between C_s and its equilibrium value, $C_{s,eq}$, and is zero when the pesticide sorbed to the substratum is in equilibrium with the pesticide dissolved in water. Equation 3 and 4 were integrated in MATLAB and simultaneously solved at each time step (Version 7.4.0.287. 2007. Matlab User's guide: Programming. The Mathworks Inc., Natick, MA, USA) with the following boundary conditions: C_s (t = 0) = 0 and C_l (t = 0) = C_{ini}.

$C_{s,eq}$ was calculated from the equilibrium concentration after 72 *h* using equation (3.1) and thus the only unknown parameter is the value of α.

The sorption coefficient or distribution coefficient K_d (*L* kg^{-1}) at both concentrations was calculated as follows:

$$K_d = \frac{C_{s,eq}}{C_{l,eq}} \tag{3.5}$$

in which $C_{s,eq}$ equals the equilibrium sorbed concentration (*mg* kg^{-1}) and $C_{l,eq}$ the equilibrium liquid concentration (*mg* L^{-1}).

3.3.4.2 Sorption isotherms

Sorption isotherms were obtained by plotting the amount of pesticide sorbed by the substratum (*mg* kg^{-1}) versus the respective concentration in equilibrium solution (*mg* L^{-1}). Three sorption models were fit to the respective data:

$$C_s = K_d \cdot C_l \text{ (linear equation)} \tag{3.6}$$

$$C_s = K_f \cdot C_l^n \text{ (Freundlich equation)} \tag{3.7}$$

$$C_s = \frac{K_1 \cdot K_2 \cdot C_l}{1 + K_2 \cdot C_l} \text{ (Langmuir equation)} \tag{3.8}$$

where C_s (*mg* kg^{-1}) is the amount of pesticide sorbed at equilibrium concentration C_l (*mg* L^{-1}), K_d is the liquid-solid distribution coefficient or sorption coefficient (*L* kg^{-1}) , K_f (*L* kg^{-1}) and K_2 (*L* kg^{-1}) represent the sorption capacity for the Freundlich and Langmuir equations respectively, *n* and K_1 (*g* kg^{-1}) are constants reflecting the sorption strength or intensity[97]. After log linearization:

$$\log C_s = \log K_f + n \log C_l \tag{3.9}$$

Freundlich parameters K_f and *n* values were determined from the curve obtained after linear regression of log C_s versus log C_l (intersection with Y-axis and slope, respectively). Langmuir parameters were determined after inversion of the equation:

$$\frac{1}{C_s} = \frac{1}{K_1 \cdot K_2} \cdot \frac{1}{C_l} + \frac{1}{K_1} \tag{3.10}$$

where $1/K_1K_2$ is the slope and $1/K_1$, the intercept of the resulting straight line. C-normalized partitioning coefficients (K_{oc}) normalized to the organic carbon content (OC) were calculated according to the equation by Hamaker and Thompson[94].

3.3.5 Influence of formulated pesticides on the sorption coefficient

A comparison between the sorption behavior of the formulated and technical product was carried out for isoproturon and bentazone on the substratum garden waste compost. Sorption trials performed were identical to the batch equilibrium technique described above. For isoproturon a comparison was carried out between technical isoproturon (98%, Bayer Crop Science, Monheim, Germany) and the formulation Isoguard 83 WG (83% isoproturon, Gharda Chemicals Ltd.). Technical bentazone (98.4% BASF, Limburgerhof, Germany) was compared with the formulation Basagran SG (87% bentazon, BASF Belgium S.A.).

3.3.6 Sorption coefficient of a mixture of substratums

All sorption coefficients were determined on individual substratums. However, to test whether the sorption coefficient of a mixture of substratums is the sum of the sorption coefficients determined for the individual substratums, batch sorption experiments were carried out with each pesticide at a concentration of 10 $mg\ L^{-1}$ on a mixture of straw, peat mix and the sandy loam soil (2:1:1), with a solid-liquid ratio of 1:10. These values were compared with the sum of the sorption coefficients determined for the individual substratums at a concentration of 10 $mg\ L^{-1}$.

3.3.7 Competitive sorption

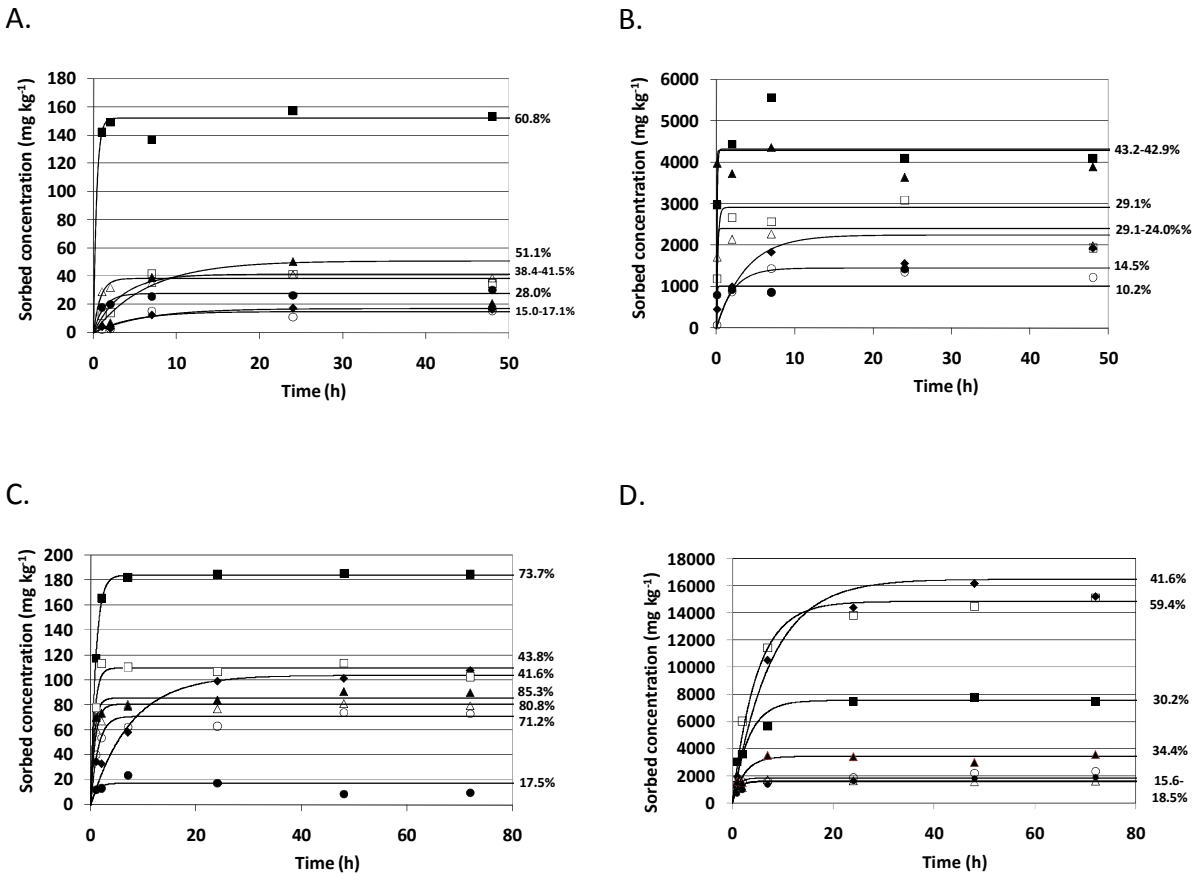
A mixture of pesticides (metalaxyl, linuron, bentazone and isoproturon) was added to garden waste compost at concentrations of 0.1, 1, 5, 10 and 50 $mg\ L^{-1}$ in a solid-liquid ratio of 1:10. The obtained Freundlich parameters were compared with Freundlich parameters of sorption of the individual pesticide on garden waste compost at the same concentrations to test whether mutual competition for sorption places exists between pesticides.

3.4 Results and discussion

3.4.1 Sorption kinetics

3.4.1.1 Determining the first-order kinetic constant α by inverse Modeling

Measured pesticide concentrations for each of the substratums and pesticides at both initial concentrations are plotted in function of the incubation time (Figure 3.1). The model elaborated in equation 3.1-3.5 was fitted to the measured time series of pesticide concentrations for all substratum/pesticide combinations at both initial pesticide concentrations. The kinetic parameter α was inversely optimized on the time series data using the Levenberg-Marquardt algorithm available in the MATLAB optimization toolbox. The solid line through each set of data points represents the optimal fit of the model to the observed data and corresponds to an optimal value of α. The corresponding determination coefficients R^2 are presented in Table 3.1, with an average of 0.83, indicating an overall good model fit.



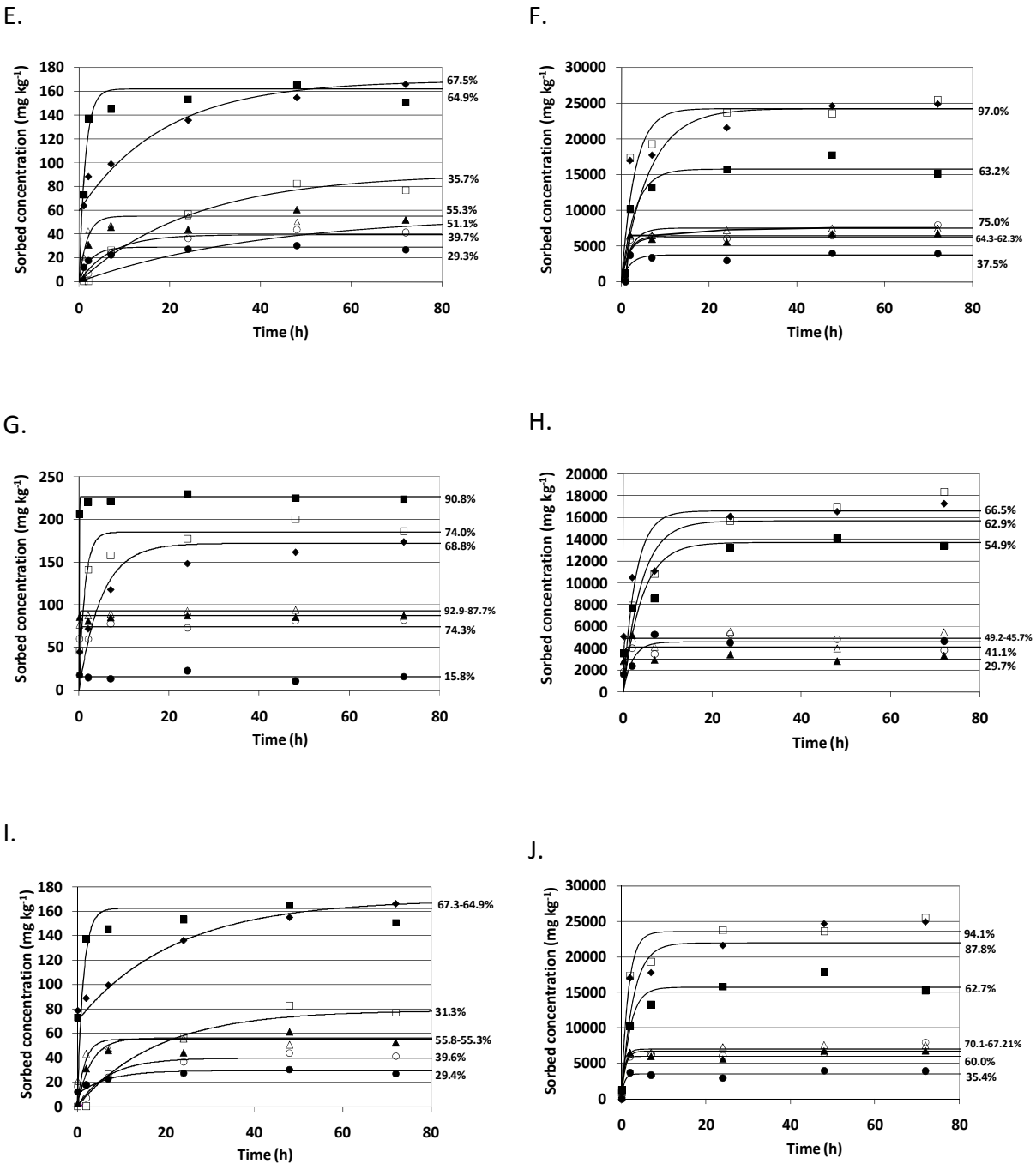


Figure 3.1: Experimental (indicated with the geometric symbols) and calculated (———) sorption kinetics of metalaxyl (A,B), isoproturon (C,D), lenacil (E,F), linuron (G,H), isoxaben (I, J) for the different substratums: ■ = peat mix, Δ = garden waste compost, ▲ = dried cow manure, ♦ = coconut chips, ○ = willow chopping, ● = sandy loam soil, □ = straw at 10 mg L^{-1} (A, C, E, G, I) and 1000 mg L^{-1} (B, D, F, H, J) as initial pesticide concentrations. The sorbed fraction (%) of the pesticides onto the substratum is indicated at the right Y-axes.

An initial steep increase in sorbed pesticide concentration was observed in all cases with 30-99% of the equilibrium concentration adsorbed after two hours. Fast initial sorption is followed by a slower sorption rate to finally reach equilibrium. Thus equilibrium between pesticides in solution and pesticides adsorbed to particles does not occur instantaneously. Overall, equilibrium was reached within 24 *h* for most pesticide-substratum combinations. This is in accordance with previous results reported by Hance[95] where equilibrium was established in a period of 24 *h* or less for monuron, linuron, atrazine and chlorpropham on a variety of adsorbents and by Kumar *et al.*[102] for endosulfan on four Indian soils (clayey soil, red soil, composted soil and sandy soil).

Table 3.1: Determination coefficients of the fitted curve to the observations.

	C_{ini}: 10 *mg* L^{-1}						
	Willow chopping	**Peat mix**	**Straw**	**Coconut chips**	**Cow manure**	**Garden waste compost**	**Sandy loam soil**
Lenacil	0.88	0.89	0.98	0.97	0.60	0.90	0.91
Linuron	0.59	0.91	0.96	0.99	0.98	0.89	0.62
Isoxaben	0.90	0.94	0.83	0.92	0.20	0.91	0.23
Isoproturon	0.89	0.99	0.94	0.97	0.67	0.85	0.19
Metalaxyl	0.79	0.57	0.86	0.96	0.55	0.78	0.80
	C_{ini}: 1000 *mg* L^{-1}						
	Willow chopping	**Peat mix**	**Straw**	**Coconut chips**	**Cow manure**	**Garden waste compost**	**Sandy loam soil**
Lenacil	0.94	0.97	0.96	0.91	0.91	0.95	0.88
Linuron	0.83	0.95	0.96	0.92	0.31	0.78	0.92
Isoxaben	0.96	0.98	0.98	0.95	0.98	0.99	0.96
Isoproturon	0.72	0.93	1.00	0.99	0.87	0.78	0.82
Metalaxyl	0.99	0.75	0.84	0.75	0.82	0.79	0.33

Optimal α values as a result of the inverse modeling approach are presented in Table 3.2. Comparing the α values reveals that isoxaben and linuron, pesticides with a high K_{oc} value, sorb faster in comparison to lenacil, isoproturon and metalaxyl at a concentration of 1000 *mg* L^{-1} and for linuron also at 10 *mg* L^{-1}. This suggests that in general strongly sorbing pesticides (high K_{oc} values) exhibit a fast kinetic sorbing process (high α values).

With respect to the substratums, garden waste compost and cow manure appear to have the highest α values for most pesticides. Thus sorption of the studied pesticides will achieve

equilibrium fast in case of the garden waste compost and the cow manure. In contrast, coconut chips will sorb pesticides slower. It is generally accepted that organic matter is the major constituent determining the extent of pesticide sorption[94,103,104]. However, when comparing the rate of sorption of the pesticides on the different substratums tested in this study, a high organic matter content does not necessarily seem to coincide with fast sorption kinetics. No correlation was found between the organic matter content of the substratums and the corresponding α values. This is reflected for example in the difference in sorption kinetics between the coconut chips (slow sorption) and the peat mix (fast sorption) although both substratums have high organic matter contents. Therefore it could be concluded that not only the amount of organic matter influences the sorption rate, but that other factors such as the type of organic matter, the specific surface area and the particle size of the substratum may contribute to this process. Coconut chips are much coarser than garden waste compost and cow manure. Therefore diffusion of the pesticide molecules onto the specific surface of the substratums will occur slower than in the garden waste compost and cow manure.

Table 3.2: Determined values of sorption rate constants α associated with the sorption of five pesticides on seven substratums at an initial concentration of 10 and 1000 mg L^{-1}

Substratums	Lenacil		Isoproturon		Metalaxyl		Isoxaben		Linuron	
	10	**1000**	**10**	**1000**	**10**	**1000**	**10**	**1000**	**10**	**1000**
Coconut chips	0.05	0.16	0.13	0.13	0.16	0.36	0.19	0.27	0.20	0.35
Dried cow manure	0.25	0.55	1.45	0.41	0.15	1.55	0.32	9.28	9.69	9.59
Garden waste compost	0.56	0.44	1.18	0.92	1.16	1.10	0.56	7.13	8.28	5.74
Peat mix	0.70	0.31	1.04	0.31	2.35	0.32	0.70	6.88	9.07	0.23
Sandy loam soil	0.39	0.49	0.94	0.21	0.79	1.34	0.15	7.89	10.79	0.46
Straw	0.02	0.32	1.38	0.98	0.29	0.60	0.06	4.10	0.75	0.24
Willow chopping	0.14	0.55	0.72	0.84	0.21	1.01	0.14	0.46	8.07	4.48

Substratums for which a high α value was found are peat mix and the sandy loam soil. Peat mix has a high organic matter content and a fine structure compared to e.g. coco chips. The sandy loam soil has a low organic matter content, but appears to sorb pesticides fast. The CEC, which is highly correlated with the surface area, was fairly low for this substratum. Hence, fast sorption could not be attributed to a higher surface area. Straw and willow chopping have an average to low α value. Straw and willow chopping both contain a substantial amount of organic matter, but their coarse structure makes diffusion of the pesticide molecules slow.

3.4.1.2 Influence of α on the pesticide leaching through a biopurification system

To evaluate the effect of the differences in optimized α values presented in Table 3.2 on the leaching of pesticides through a hypothetical biopurification system, a HYDRUS-1D model[105] was applied to simulate pesticide transport. Small rate constants indicate a slow sorption process and could therefore increase the risk of pesticide leaching. The biopurification system which was simulated was a biofilter. The leaching of a pesticide in this system was simulated in one biofilter unit with a height of 1m. For the sake of simplicity, the hypothetical porous medium was assumed to be saturated without taking the air fraction into account. With this assumption, the selected hydraulic conductivity K_s was set equal to the applied flux, the dispersivity λ was estimated as 1/10th of the column length and the estimated volumetric saturated moisture content θ_s was 60%. These parameters were fixed for all simulations. The leaching of the pesticides linuron (immobile pesticide) and lenacil (mobile pesticide) was simulated when added at a concentration of 10 $mg\ L^{-1}$ and with a first-order degradation constant $\mu_{solid\ phase}$ set at 0.0146 d^{-1} and 0.0039 d^{-1} respectively and $\mu_{liquid\ phase}$ set at 0.0053 d^{-1} and 0.0076 d^{-1} respectively (these values were based on mineralization experiments performed by Sniegowski, K. (personal communication). The matrix of the biofilter consisted of 25% peat mix, 25% sandy loam soil and 50% straw. The sorption coefficients K_d for linuron and lenacil in this mixture were respectively 97.9 and 21.7 $L\ kg^{-1}$ and were calculated as the weighted mean of the individual pesticide-substratum K_d values (these values were obtained by calculating the K_d value at equilibrium for each pesticide–substratum combination).

A. B.

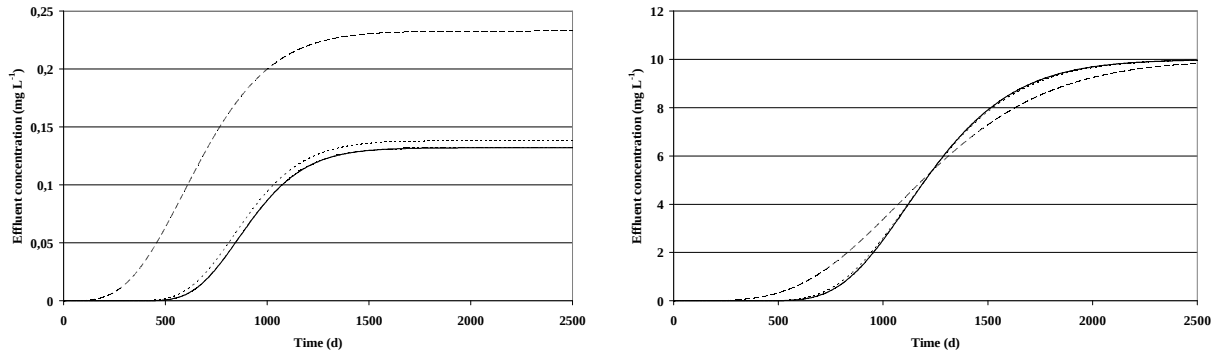
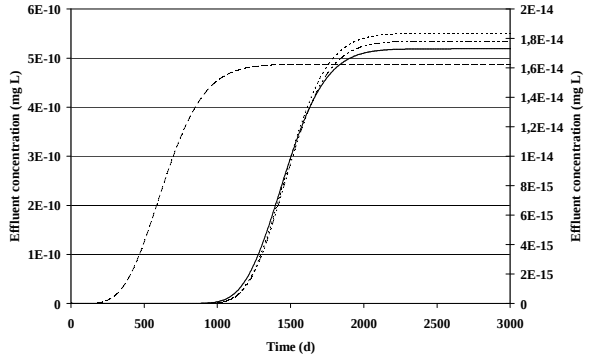


Figure 3.2: Influence of the sorption rate constant α on the shape of the breakthrough curve of lenacil in a hypothetical biopurification system: A. with degradation B. without degradation *(——— = instantaneous equilibrium, ····· = $\alpha_{mixture}$ = 0.28, – – – = α_{max} = 10.79, — — · = α_{min} = 0.02).*

A.



B.

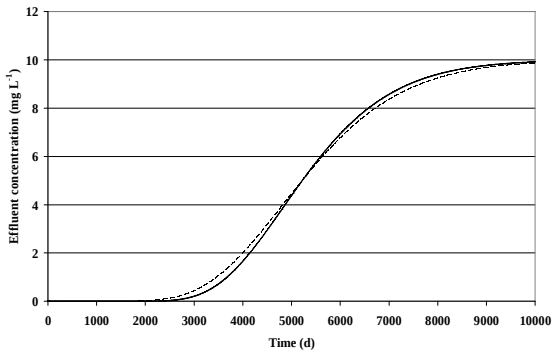


Figure 3.3: Influence of the sorption rate constant α on the shape of the breakthrough curve of linuron in a hypothetical biopurification system: A. with degradation B. without degradation *(——— = instantaneous equilibrium, ····· = $\alpha_{mixture}$ = 5.34, – – – = α_{max} = 10.79, — — · = α_{min} = 0.02).*

The applied flux on top of the system was set at 1 *cm* d^{-1} and was added as a step input, as frequently applied in such a system. Four scenarios with and without degradation were simulated: a) non-equilibrium with the kinetic constant of the simulated mixture, *i.e.* α = 5.34 for linuron and α = 0.28 for lenacil, b) a low α value (α = 0.02), c) a high α value (α = 10.79) (Table 3.2) and d) instantaneous equilibrium. The breakthrough curves are presented in Figure 3.2 and Figure 3.3 for respectively lenacil and linuron. For linuron, the breakthrough curves with degradation assuming instantaneous equilibrium, α = 5.34 and α = 10.79 are plotted against a secondary Y-axis in Figure 3.3A.

Breakthrough occurs clearly faster for lenacil compared to linuron. Linuron has a higher K_d value than lenacil, which causes a stronger retention into the matrix of the system. Furthermore, breakthrough occurs faster when the low rate constant (α = 0.02) was taken into account, which means that the resident time of lenacil and linuron in the system will be lower than when instantaneous equilibrium is assumed. The breakthrough curves of linuron and lenacil were in case of a high rate constant (α = 10.79) very similar to those obtained assuming instantaneous equilibrium. The same is true when a rate constant associated with a classical biomix (α = 0.28 for lenacil and α = 5.34 for linuron) is assumed. This also resulted in breakthrough curves comparable to the situation were instantaneous equilibrium is assumed. Hence, at fairly 'realistic' values, the sorption rate constant had a minor influence on pesticide breakthrough compared with instantaneous sorption.

In general, when degradation is taken into account, a lower effluent concentration is achieved. Lenacil, which is a more persistent, but more mobile pesticide than linuron will leave the system at a higher concentration than linuron, due to the shorter resident time. However, this effluent concentration also depends on the α value associated with the pesticide-substratum combination. For lenacil, the effluent concentration in a system with a low sorption rate constant (α = 0.02) was 1.8 times higher compared to an instantaneous equilibrium situation. A slower degradation of the pesticides in the system occurs due to a decreased contact time between the pesticide and pesticide degrading microorganisms

present in the system. A high α value (α = 10.79), representing a fast sorption process, shows a breakthrough curve with a similar pattern to that assuming instantaneous equilibrium and thus significant degradation occurred. The effluent concentration of linuron in a system with a low rate constant (α = 0.02) was almost 30000 times higher than in an instantaneous equilibrium situation. However, the effluent concentrations of linuron are extremely low (< $6 \cdot 10^{-10}$ *mg* L^{-1}), which makes the difference between a slow and fast sorption process almost negligible. Consequently, the influence of the rate constant α on the effluent concentration depends on the mobility of the pesticide. The effluent concentration is more subjective to changes in the rate constant α when it concerns a pesticide with a relatively high mobility such as lenacil. Due to a lower resident time, pesticides will have less time to be degraded or retained. Therefore, for mobile pesticides, sorption should occur fast which leads to a higher retention and probable higher degradation of the pesticide. As such, kinetic rate constants are important to determine the optimal substratum combination, which ensures a high retention and degradation.

3.4.2 Sorption isotherms

3.4.2.1 Sorption isotherm models

To determine the ideal model for the obtained sorption data, three sorption models were fitted to the experimental data (Table 3.3). The determination coefficients (R^2) for the linear sorption model ranged from 0.23 to 0.99, for the Freundlich equation from 0.85 to 1.00 and for the Langmuir equation from 0.01 to 1.00. A consistently good fit of the linear model to the sorption data on all substratums could only be observed for lenacil. Thus, the obtained sorption isotherms were in general non-linear. According to Spurlock and Biggar[106], this lack of linearity is due to specific interactions between polar groups of the pesticide and the organic material of the substratum.

The Langmuir equation only showed a good fit for the sorption of linuron and lenacil for all substratums. This is however attributed to the large range of pesticide concentrations used. The sorption isotherm was mainly constructed between two clusters of points. Due to the inversion of C_s and C_l and due to the large range of pesticide concentrations with a majority of high concentrations, a cluster of observation points is present near point zero, while only one or two points (of the initial concentrations of 1 and 10 *mg* L^{-1}) are present at higher points on the X and Y-axes. Moreover, in Table 3.3 negative values could be observed for K_1 and K_2 which is improbable[97]. It indicates that this model failed to describe the sorption of the tested pesticides on the organic substratums studied. Therefore the sorption isotherms will further be discussed in function of the Freundlich equation, which best described the observed data.

The Freundlich equation has mainly been used to describe the sorption behavior of pesticides[95,107-112]. Freundlich sorption isotherms for the six pesticides on seven substratums used in a biopurification system are shown in Figure 3.4. The small size of the error bars (± standard deviation) indicates good reproducibility of replicate samples and hence good overall precision of the batch technique.

Table 3.3: Sorption parameters K_f (L kg^{-1}), n(-), K_1 (g kg^{-1}), K_2 (L kg^{-1}), K_d (L kg^{-1}) for the pesticides studied on relevant organic substratums.

Substratum	Sorption model	Parameter	Metalaxyl	Isoproturon	Linuron	Isoxaben	Bentazone	Lenacil
Coco chips	Freundlich	K_f	10.66ab[a]	9.22b	51.01bc	76.79b	7.08bc	83.48c
		n	0.98	1.17	0.85	0.83	0.79	0.89
		R^2	0.99	1.00	0.95	0.99	0.99	0.99
	Langmuir	K_1	-121.06a	515.1691ab	1384.15ab	340.33ab	0.15a	2502.48ab
		K_2	-0.05a	-0.00c	0.05b	3.33a	-1.79a	0.11a
		R^2	1.00	1.00	1.00	0.98	0.06	1.00
	Linear	K_d	7.51ab	31.38b	9.28a	6.97a	1.62a	24.57b
		R^2	0.97	0.99	0.86	0.93	0.71	0.98
Garden waste compost	Freundlich	K_f	16.59c	27.08c	80.55cd	53.26ab	2.45ab	65.95bc
		n	0.81	0.61	0.58	0.39	0.78	0.65
		R^2	1.00	0.98	0.95	0.85	0.98	0.97
	Langmuir	K_1	748.64a	2877.94bc	-1298.87ab	21856.60c	54.67a	227.83ab
		K_2	0.03a	0.01c	-0.12ab	0.06a	0.08ab	1.96a
		R^2	0.97	1.00	1.00	0.97	0.01	0.97
	Linear	K_d	3.24ab	1.22a	1.77a	5.68a	0.66a	2.58a
		R^2	0.89	0.97	0.70	0.23	0.88	0.93
Sandy loam soil	Freundlich	K_f	6.91a	2.12a	2.86a	11.47a	0.57a	5.57a
		n	0.74	1.09	0.86	0.63	0.91	0.83
		R^2	1.00	0.96	0.86	0.94	0.95	0.99
	Langmuir	K_1	351.95a	7.41ab	67.86ab	-39.37ab	6.67a	482.66ab
		K_2	0.02a	-0.12a	0.05b	5.09a	-0.24ab	0.01a
		R^2	1.00	0.74	1.00	0.99	0.41	1.00
	Linear	K_d	0.80a	1.25a	0.69a	0.46a	0.70a	1.17a
		R^2	0.92	0.82	0.30	0.56	0.79	0.92
Cow manure	Freundlich	K_f	4.25a	34.87c	47.67bc	40.38ab	4.36abc	18.98ab
		n	1.20	0.71	0.60	0.67	0.79	0.86
		R^2	0.99	0.98	0.97	0.97	0.96	1.00
	Langmuir	K_1	-9813.60a	-560.08ab	1201.80ab	-7881.34a	2598.58ab	8740.99ab
		K_2	-0.02a	-0.08ab	-0.01ab	-0.02a	0.34ab	5.62a
		R^2	0.01	1.00	1.00	0.92	0.83	0.99
	Linear	K_d	13.29b	1.95	1.85a	2.35a	1.08a	7.27ab
		R^2	0.96	0.47	0.81	0.98	0.96	0.96
Peat mix	Freundlich	K_f	58.83d	57.27d	192.57e	198.82c	9.27c	156.75d
		n	0.74	0.77	0.63	0.59	0.76	0.68
		R^2	0.98	0.99	0.97	0.98	0.92	0.99
	Langmuir	K_1	324.50a	6172.96c	-8633.71a	6278.75abc	2063.51ab	2707.05b
		K_2	-0.06a	0.01c	-0.16a	4.82a	1.62b	0.68a
		R^2	0.94	1.00	0.99	0.97	0.52	0.99
	Linear	K_d	7.69ab	10.30a	4.98a	9.86a	2.66b	9.45ab
		R^2	0.96	0.99	0.65	0.88	0.88	0.97
Straw	Freundlich	K_f	6.58a	7.91ab	95.28d	242.04c	2.54ab	101.32c
		n	1.01	1.08	0.81	0.57	0.89	0.83
		R^2	1.00	0.96	0.99	0.92	0.90	0.95
	Langmuir	K_1	-1838.39a	-715.14a	37438.82c	259.90ab	-592.18a	-17898.74a
		K_2	-0.01a	-0.01c	0.00ab	4.09a	-0.00ab	-0.00a
		R^2	0.99	1.00	0.99	0.60	0.11	0.95
	Linear	K_d	9.79ab	2.05a	8.63a	3.68a	0.75a	8.35ab
		R^2	0.94	0.69	0.80	0.39	0.97	0.96
Willow chopping	Freundlich	K_f	2.29a	8.98b	29.65ab	56.08ab	2.20ab	31.38abc
		n	1.01	0.79	0.74	0.54	0.76	0.75
		R^2	0.97	0.98	0.98	0.99	0.93	0.96
	Langmuir	K_1	5.57a	-225.90ab	8520.96b	12163.39bc	6956.17b	9374.85ab
		K_2	4.38a	-0.03bc	0.01ab	0.01a	-0.00ab	0.00a
		R^2	0.96	0.99	1.00	0.97	0.38	0.93
	Linear	K_d	1.72ab	8.65b	6.12a	3.01a	0.85a	15.82ab
		R^2	0.79	0.99	0.73	0.92	0.91	0.90

[a] Different letters in the same column indicate significant differences ($p < 0.05$) by Duncan test.

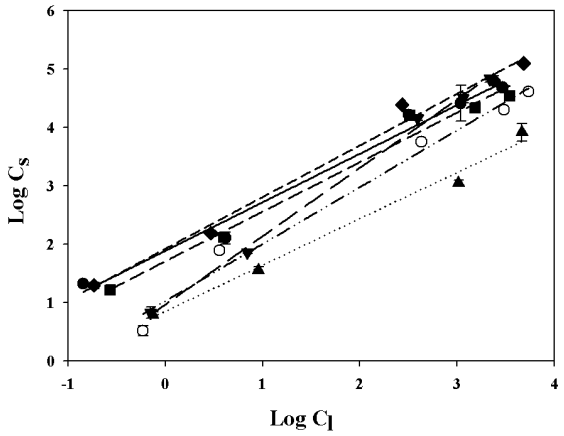
3.4.2.2 Freundlich parameters

The Freundlich parameters K_f and n for the different pesticide-substratum combinations are presented in Table 3.3. Based on the n value, isotherms can be classified as an L, S or C-type according to Giles *et al.*[113]. In general, it was observed that isotherms were of the L-type (n < 1). However for some pesticide-substratum combinations S-type (n > 1) isotherms were observed (*e.g.* metalaxyl in contact with cow manure, straw and willow chopping and isoproturon on the substratums coco chips, sandy loam soil, and straw). This indicates that the mutual interaction between metalaxyl or isoproturon molecules is higher than the sorbate-sorbent interaction at high concentrations. A similar S-type isotherm has also been reported for linuron[114], metobromuron, aclonifen[115], MCPA[116], chlorpyrifos[117], alachlor, metolachlor, and chlorothalonil[118] on different types of soil. The n value was in general lowest for the pesticides isoxaben and linuron. This indicates a high affinity between the pesticide and the substratum. This high affinity correlates with their relatively high K_{oc} value.

The position of the sorption curve in the graph is an indication of the sorption ability of a certain pesticide on a substratum. The higher the curve is located on the Y-axis, the more pesticide is sorbed. The low sorption of bentazon (pKa = 3.3) is clear for all the substratums (Figure 3.4). The pH of the substratums varied from 5 to 7.7. The Freundlich parameters of bentazone on these substratums are not significantly different, thus the influence of pH on bentazone sorption is negligible. The curve obtained for this pesticide with all sorbents is below the sorption isotherms obtained for the other pesticide-substratum combinations.

In general, non-ionic pesticides are relatively less mobile than ionic pesticides, such as bentazon. Non-ionic pesticides are hydrophobic with a low water solubility and consequently their affinity for organic matter is high[109]. Linuron, lenacil and isoxaben were most strongly sorbed to most of the substratums. Pesticides with intermediate sorption capacity were metalaxyl and isoproturon. This is more or less in accordance with their reported K_{oc} values. Freundlich parameters reported in literature are mostly based on interactions with soil. Therefore a comparison between the observed and reported values was made for soils with similar texture to the studied sandy loam soil. This comparison pointed out that for metalaxyl similar values were found by Sharma & Awasthi[119] who found a K_f = 10.65 $L\ kg^{-1}$ and n = 0.615 in a concentration range of 0.3 to 20 $mg\ L^{-1}$. For isoproturon, K_f values of 1.3-3.6 $L\ kg^{-1}$ and n values of 0.73-0.83 have been reported[111,120,121] in a concentration range of 0.08 to 20 $mg\ L^{-1}$. A K_f value of 5.77 $L\ kg^{-1}$ and n value of 0.76 were previously reported for linuron in a concentration range of 1 to 10 $mg\ L^{-1}$. These values are in the same range of the observed values considering the slight difference in soil composition and pesticide concentration range studied[122]. For lenacil there were no similarities between the observed and reported Freundlich parameters due to the use of soil types with different composition. Previously reported K_f and n values of the sorption of isoxaben were respectively 13.8 $L\ kg^{-1}$ and 1.05 in a tested concentration range of 0 to 1 $mg\ L^{-1}$[123], which is in line with our observations. Previously reported K_f values of bentazon ranged from 0.03-1.46 $L\ kg^{-1}$ while n values ranged from 0.88 to 1.06 in a concentration range of 1 to 120 $mg\ L^{-1}$. The higher K_f value obtained for bentazon in this study can be explained by the higher organic carbon content of the studied substratums .

A.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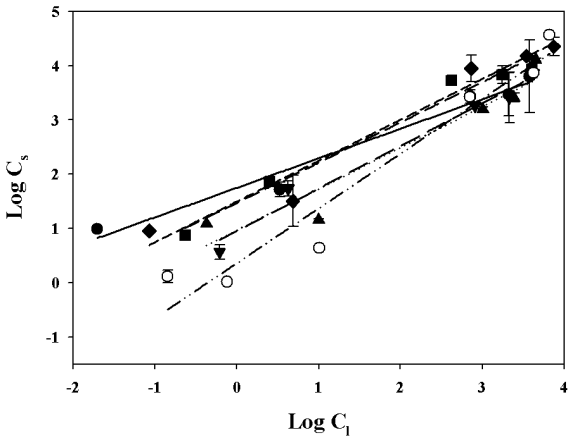
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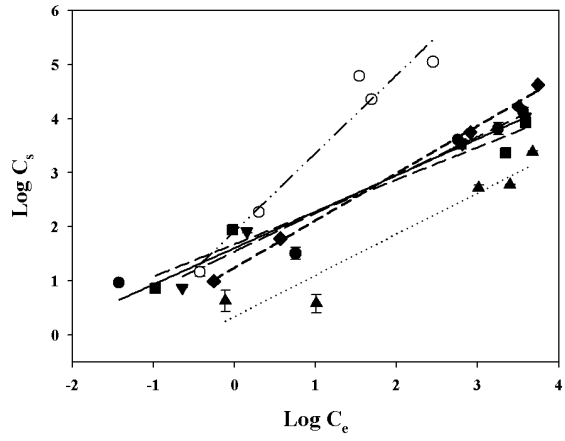
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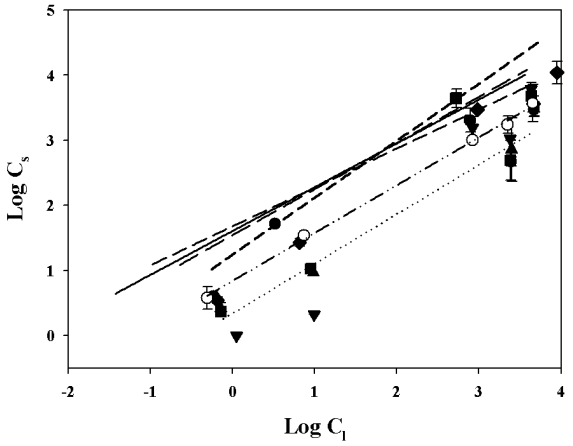
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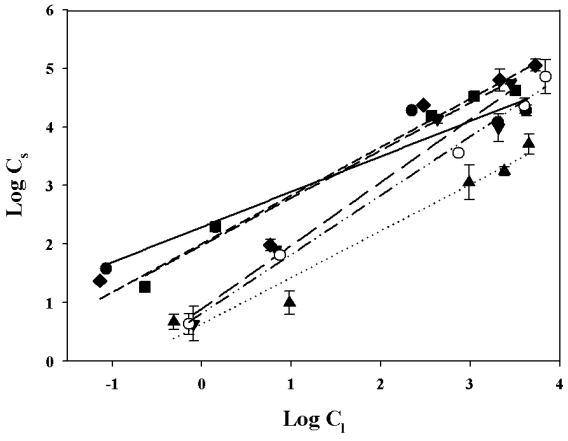
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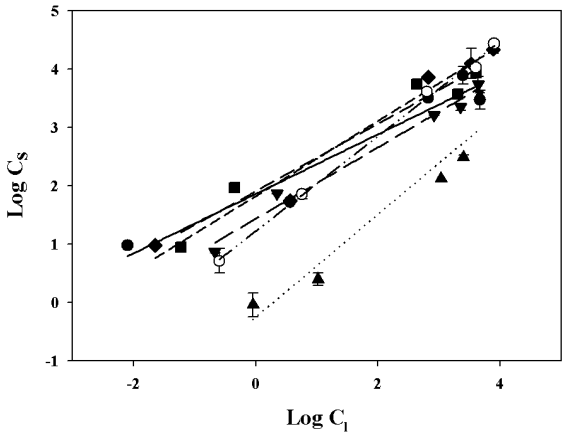
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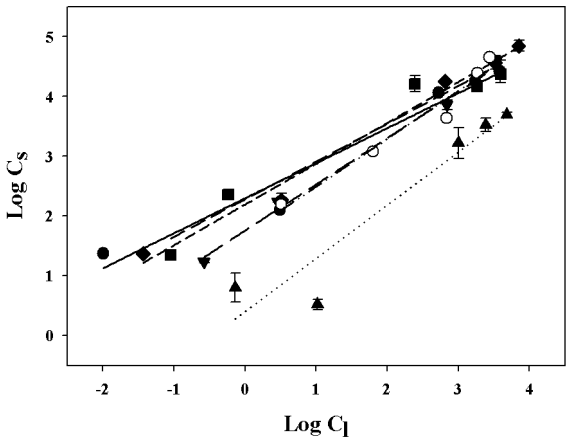

Figure 3.4: Sorption isotherms, presented as the logarithm of the sorbed concentration C_s (log mg kg^{-1}*) versus the logarithm of the concentration in the liquid phase C_l (log* mg L^{-1}*) obtained for isoxaben (● , ———), isoproturon (▼ , — —), linuron (■ , — —), lenacil (◆, — — —), bentazon (▲, ·····) and metalaxyl (○ , — ·· —) when in contact with A. coco chips, B. willow chopping, C. cow manure, D. sandy loam soil E. straw, F. garden waste compost, G. peat mix (*n=*2) (2 x standard error ≤ symbol height unless otherwise indicated).*

After comparison of the K_f values between substratums, it could be observed that the use of sandy loam soil results in general into the lowest value compared to other substratums (Table 3.3). This is in contrast to the use of peat mix which results in a significantly higher K_f value for 4 out of 6 pesticides. No significant differences could be observed between straw and peat mix for the sorption of isoxaben and between coco chips, cow manure and peat mix for the sorption of bentazon. In general, substratums were classified in different groups according their sorption capacity: peat mix > compost, coco chips, straw > cow manure, willow chopping > sandy loam soil.

It is generally accepted that the organic matter content is the major factor determining the extent of sorption[94,103,104]. However in this case, when comparing the sorption capacity of different substratums, high organic matter content did not necessarily coincide with high K_f values. Peat mix has a similar organic matter content as willow chopping, but has a higher sorption capacity. However peat mix is more decomposed which leads to an increasing amount of aromatic compounds in the organic matter while ageing, and could therefore lead to an increase in the sorption capacity[124].

The C-normalized partitioning coefficient (K_{oc}) is generally assumed to be constant for a particular chemical when sorption is related to the 'quantity' of organic carbon (OC) in the soil, yet it is now widely recognized that chemical sorption is also affected by the 'quality' or nature of the OC[125-127]. As the isotherms were not linear, the K_{oc} values had to be calculated from a specific K_d value calculated for one pesticide concentration. The K_{oc} value was determined by dividing K_d with the %OC. The K_d was calculated as the ratio between C_s (calculated using the Freundlich equation) and C_l for a low initial concentration, C_{ini} = 10 $mg\ L^{-1}$. This concentration was chosen as intermediate between a low and high contamination.

Thus this K_{oc} values can not be generalized but only indicate differences in sorption between substratums normalized to the organic carbon content at low pesticide contaminations.

The K_{oc} values associated with the sorption of the 6 studied pesticides on the 7 substratums are presented in Table 3.4. The K_{oc} value did not reduce the variation among different substratums. The ratio between the lowest and the highest K_{oc} value still varied from 6 to 23 for the different pesticides (excluding the K_{oc} value of the sandy loam soil, due to much lower % OC compared to the organic substratums). This indicates that other parameters will also influence sorption of the studied pesticides. However, for peat mix, which showed a high sorption capacity, a normalization of the K_d value to the organic carbon content significantly reduced the variation in this value, indicating that high sorption on this substratum will mainly be attributed to the high organic carbon content.

Table 3.4: Calculated K_{oc} (L kg^{-1}) values of the studied pesticides for the seven substratums

Substratum	Metalaxyl	Linuron	Lenacil	Isoxaben	Isoproturon	Bentazone
Coco chips	23.85	114.14	186.80	171.83	20.63	15.84
Garden waste compost	59.59	289.33	236.89	191.31	97.27	8.80
Cow manure	11.31	126.82	50.49	107.42	92.76	11.60
Peat mix	123.57	404.47	329.24	417.60	121.24	19.47
Straw	15.54	225.04	239.30	571.66	18.68	6.00
Willow chopping	5.36	69.34	73.39	131.15	21.00	5.14
Sandy loam soil	759.34	314.29	612.09	1260.44	232.97	62.64

To determine the factors interfering in pesticide sorption, Pearson's correlation coefficients (r) between substratum characteristics (Table 2.1) and the sorption coefficient K_d (determined for each initial concentration) were calculated. For the majority of pesticides a significant positive correlation ($p < 0.05$) was found between the sorption coefficient and the organic carbon content (OC), CaO, and the cation exchange capacity (CEC) (Table 3.5). The positive effect of calcium on sorption of paraquat was previously described by Amondham *et al.*[128]. A possible explanation of the enhanced sorption by CaO and the CEC could be the formation of complexes with the added pesticides.

Table 3.5: Pearson's correlation coefficients (r) between substratum characteristics and the sorption coefficient K_d for all studied pesticides

	metalaxyl	isoproturon	linuron	isoxaben	bentazone	lenacil
pH	0,153	0,090	0,087	0,009	-0,023	0,047
OC	0,249*	0,652*	0,419*	0,320*	0,313*	-0,026
P_2O_5	0,232	0,095	-0,080	-0,081	-0,003	0,306*
K_2O	0,183	0,159	-0,100	-0,168	-0,072	0,153
CaO	0,213	0,639*	0,440*	0,373*	0,308*	-0,048
MgO	0,236	0,111	-0,036	-0,033	0,030	0,304*
Na	0,222	0,249	-0,057	-0,098	-0,024	0,083
CEC	0,323*	0,747*	0,427*	0,346*	0,310*	0,018
Specific density	-0,203	0,329*	-0,214	-0,094	-0,113	-0,051

*correlation is significant at the 0.05 level (2-tailed)

3.4.3 Influence of formulated pesticides on the sorption coefficient

Formulated pesticides consist of an active substance and adjuvants, such as oils, surfactants, solvents, polymers, etc. It was previously reported that sorption of a formulated pesticide can be lower than sorption of the active substance[129]. The calculated sorption coefficients of formulated and technical linuron and isoproturon are presented in Table 3.6. No significant differences could be observed in K_f values between the formulated and the technical product of both pesticides. The composition of the formulations studied is unknown due to patent protection, but most likely as these formulations are both water dispersable or soluble granulates, they will contain surfactants. Similar results have been observed for organophosphate insecticides in combination with non-ionic (Tween 80) and anionic (Aerosol 22) surfactants[130].

Table 3.6: Freundlich parameters calculated for technical and formulated isoproturon and bentazon sorbed on garden waste compost (±95% confidence interval (n=3)).

		K_f ($L\ kg^{-1}$)	*n*
Isoproturon	**Technical**	30.93 ± 0.25	0.84 ± 0.08
	Formulated	30.12 ± 2.61	0.92 ± 0.01
Bentazone	**Technical**	2.07 ± 0.02	1.16 ± 0.00
	Formulated	2.41 ± 0.38	1.15 ± 0.03

3.4.4 Sorption coefficient of a mixture of substratums

In this study, the sorption coefficient of each of the studied pesticides was determined on an individual substratum. However, in a biopurification system, pesticides will be in contact with a mixture of substratums. To be able to use the individual sorption coefficients to model the transport of pesticides in a biopurification system without performing batch sorption experiments on the mix of substratums, the sum of the sorption coefficients of the studied pesticides on the individual substratums should be equal to the sorption coefficient of isoproturon on a mixture of substratums, to simplify calculations. In Table 3.7 no significant differences could be observed between the mixture and the sum. Subsequently, the sorption coefficients of the individual substratums can be used to determine the sorption capacity of the total mixture.

Table 3.7: Sorption coefficients as a result of the sum of the individual sorption coefficients K_d (sum) (L kg^{-1}) *and experimental sorption coefficient* K_d *(exp) (L* kg^{-1}*) on a mixture of straw (50%), peat mix (25%) and sandy loam soil (25%) (±95% confidence interval (n=3)).*

	Bentazone	Isoproturon	Isoxaben	Lenacil	Linuron	Metalaxyl
K_d **(sum)**	0.59 ± 0.15	21.03 ± 1.27	84.91 ± 8.40	15.71 ± 4.73	194.21 ± 15.32	17.70 ± 2.63
K_d **(exp)**	0.66 ± 0.18	20.02 ± 2.65	95.10 ± 4.56	14.65 ± 3.98	180.50 ± 10.79	19.65 ± 3.78

3.4.5 Competitive sorption

Pesticides differ chemically and thus, can adsorb in different ways. In a biopurification system, pesticides can be added as a mixture, not as an individual compound. The interactions between substratums and pesticides might be different when the pesticide is added in a mixture with other active ingredients. The pesticides may compete for sorption sites thus reducing their individual sorption. A decrease in sorption could provoke an increase in the leaching potential of the pesticide. Therefore in order to be able to model the transport of pesticides in the system, the sorption coefficient of pesticides in a mixture was determined. The Freundlich coefficients of metalaxyl, isoproturon, linuron and bentazon applied as a single compound or as a mixture are presented in Table 3.8.

Sorption of isoproturon, linuron and bentazon on garden waste compost was not significantly affected by the presence of the other pesticides. Metalaxyl on the other hand, has a significantly lower K_f value when it is applied in a mixture with other pesticides. Previously, a lower sorption was also observed for iprodione in combination with carbendazim[131], for dimethoate in combination with malathion, diazinon and methidation[130] and for atrazine at higher concentrations of bifenox[132], etc. A possible explanation could be the difference in molecular weight of the pesticides. When a concentration of 10 *mg* L^{-1} is applied, 0.0358 *mM* metalaxyl, 0.0485 *m*M isoproturon, 0.0401 *mM* linuron and 0.0416 *mM* bentazon is present. This means that more isoproturon, linuron and bentazon molecules are present in the solution which increases the chance of finding sorption places and thus could

lead to a decrease in sorption of metalaxyl. Nonetheless, differences in molecular weight are small.

Table 3.8: Freundlich parameters obtained with the sorption of an individual application or a mixture of metalaxyl, isoproturon, linuron and bentazon on garden waste compost (±95% confidence interval (n=3)).

	K_f (L kg^{-1})		n	
	Individual	**Mixture**	**Individual**	**Mixture**
Metalaxyl	14.24 ± 1.96	7.11 ± 2.30	0.86 ± 0.06	0.92 ± 0.12
Isoproturon	30.99 ± 0.25	30.76 ± 0.91	0.84 ± 0.00	0.64 ± 0.01
Linuron	89.06 ± 5.85	95.01 ± 8.07	1.27 ± 0.01	1.01 ± 0.03
Bentazon	1.10 ± 0.02	1.05 ± 0.06	1.15 ± 0.00	1.31 ± 0.03

3.5 Conclusion

As sorption is a very important process determining the efficiency of the biopurification system, it needs to be well characterized. Sorption can increase the efficiency by increasing the retention and by positively influencing degradation of *e.g.* mobile pesticides by increasing the residence time of the pesticide in the system. To optimize retention of pesticides in the system, knowledge should not only be gained on the sorption capacity of different organic substratums but also on the sorption kinetics. To study the evolution of sorption in time, expressed by the first order kinetic constant α, sorption trials were performed using different pesticides (linuron, isoproturon, metalaxyl, isoxaben, and lenacil) and substratums commonly used in a biopurification system, *i.e.*, cow manure, straw, willow chopping, soil, coconut chips, garden waste compost, and peat mix. It could be concluded that strongly sorbing pesticides such as isoxaben and linuron have a higher optimized α value, which indicates the existence of a possible correlation between the organic carbon partition coefficient K_{oc} and the kinetic constant α.

Concerning the composition of the substratums, no relation could be found between the amount of organic matter and the rate of sorption. The structure (specific surface, particle size, etc.) of the substratum material could largely influence the sorption rate. Dense material, such as coconut chips can have a high specific surface area which is however not always available, resulting in a decrease in the kinetic constant α. To conclude the part on sorption kinetics, model simulations were performed (HYDRUS-1D) in order to determine the influence of α on the fate of pesticides in biopurification systems. Differences in optimized α values could significantly influence the leaching of pesticides through biopurification systems, as low rate constants cause a faster leaching and lower degradation, which results into a decrease in efficiency of the system. For example, leaching of lenacil occurred after ± 175 *d* with a degradation of 97.7% at the lowest sorption rate constant, while leaching at the highest sorption rate constant or at instant equilibrium occurred after ± 500 *d* with a degradation of 98.6%.

To characterize sorption at equilibrium, different models were applied. The Freundlich isotherm was the best model to describe sorption of linuron, isoproturon, metalaxyl, isoxaben, bentazon and lenacil on the substratums mentioned above. Substratums could be classified in order of increasing sorption capacity: sandy loam soil < willow chopping, cow manure < straw, coco chips, compost < peat mix. Out of this ranking and with the function of each substratum in mind, it could be advisable to use peat mix as a nutrient source for micro organisms, as a substratum with a high water holding capacity and as the substratum most efficient in retaining pesticides. The use of straw or coco chips should also be included in the matrix as these substratums serve as structure elements. The difference between the two substratums is the available carbon. Straw is easily mineralized, in contrast to coco chips which could therefore be more sustainable. Willow chopping is also a structure element but is however less suitable for use due to its low sorption capacity. Finally sandy loam soil, with its very low sorptive capacity, can not be excluded as this substratum serves as the major source of possible pesticide degrading micro organisms. The quantity of soil could however be decreased to improve retention. Cow manure with an average sorption capacity is of less importance as this substratum can never be added in large amounts as this would decrease the C/N ratio drastically. The sorption capacity of the substratums was positively correlated with the organic carbon content, CaO content and the cation exchange capacity. Phenomena investigated in this study which can have an additional influence on the retention of pesticides in the biopurification system are the influence of adjuvants, competitive sorption and additivity of the sorption coefficient. The formulation or presence of adjuvants did not have a significant influence on the sorption of isoproturon and bentazon. As regards competitive sorption, it could be observed that sorption of metalaxyl was lower in combination with bentazon, linuron and isoproturon. This phenomenon can decrease the efficiency of the system if combinations of antagonistic pesticides are used. Finally, the sorption coefficient K_d was additive which makes the summation of individual sorption coefficients possible. This could facilitate the development of a model which describes transport of pesticides in biopurification systems or in soils amended with organic substratums.

Chapter 4: Pesticide retention and degradation in micro- and macro scale biopurification systems

This chapter has been compiled from:

De Wilde, T., Mertens, J., Šimůnek, J., Sniegowski, K., Ryckeboer, J., Jaeken, P. , Springael, D., Spanoghe, P. 2009. Characterizing pesticide sorption and degradation in microscale biopurification systems using column displacement experiments, Environmental Pollution, 157, 463-473.

De Wilde, T., Spanoghe, P., Mertens, J., Sniegowski, K., Ryckeboer, J., Jaeken, P. , Springael, D., 2009. Characterizing pesticide sorption and degradation in macro scale biopurification systems using column displacement experiments, Environmental Pollution, 157, 1373-1381.

4.1 Abstract

To optimize the performance of biopurification systems, knowledge of degradation and retention processes needs to be generated. Therefore, displacement experiments were carried out for four pesticides (isoproturon, bentazone, metalaxyl, linuron) in small and large columns containing different substratum mixtures. Retention of the pesticides was similar in the micro- and macrocosms, however, in some cases a slightly lower retention was observed in the macrocosms compared to the microcosms. Differences in retention between the different mixes were minimal. Moreover, the classification of the retention strength of the pesticides was identical in both micro- and macrocosms and did not depend on the used mixture: linuron > isoproturon > metalaxyl > bentazone. Monod kinetics were used to describe delayed degradation, which occurred for metalaxyl in the microcosms and for metalaxyl, isoproturon and bentazon in the macrocosms. No breakthrough of linuron was observed, thus, this pesticide was appointed as the most retained and/or degraded pesticide, followed by isoproturon, metalaxyl and bentazon. Finally, most of the matrix mixes efficient in degrading or retaining pesticides were mixes containing dried cow manure.

4.2 Introduction

As mentioned in the introduction, on-farm biopurification systems have a typical overall clean-up efficiency which exceeds 95% and often removes more than 99% of the pesticide contamination. However, at the time of publication, biopurification systems operate as a black box, since the research carried out to sufficiently understand processes taking place inside these systems is limited. In order to optimize the efficiency of these systems for a broad range of pesticides and organic substratums, the fate of pesticides and the contribution of degradation and retention processes inside these systems needs to be well characterized. Sorption or retention of six pesticides was already profoundly studied in batch experiments in Chapter 3, but these experiments did not take transport and degradation of the pesticide into account. Therefore column displacement experiments were set-up, to determine retention and degradation during transport of four previously studied pesticides (linuron, bentazone, metalaxyl and isoproturon) in systems containing different mixtures of substratums. While the transport of pesticides has been frequently evaluated in soil columns, this study used organic materials, which have not previously been characterized in detail.

In column displacement experiments a pesticide step input is applied on top of the column. This set-up generates breakthrough curves (BTCs) from which, by the use of inverse modeling, retention and degradation parameters can be determined. The one-dimensional transport model HYDRUS-1D[105] was used to identify and quantify solute transport and hydraulic parameters by inverse modeling. The advantage of column experiments is that retention and degradation, two processes which strongly interact, can be determined simultaneously. The obtained retention parameters were compared with those calculated from batch experiments (Chapter 3). Degradation of pesticides was evaluated using the first-order or Monod kinetics, the latter to relate degradation to possible microbial growth.

Column experiments were performed on a small and large scale. Large scale columns were set-up to validate the results obtained on a small scale. In addition to the column experiments, batch degradation experiments were also performed to study the influence of a continuous application of pesticides on the degradation potential of the pesticide degrading community. It has been reported by Arbeli and Fuentes[133], Racke and Coats[134] and Vischetti *et al.*[67] that repeated pesticide application could lead to the development of an adapted microbial consortium.

4.3 Materials and Methods

4.3.1 Experimental set-up

4.3.1.1 Pesticides and chemicals

The pesticides used in this study were linuron, metalaxyl, bentazone and isoproturon Technical grade metalaxyl (95.5% purity) was kindly supplied by Syngenta (Basel, Switzerland), technical grade linuron (97.7% purity) by Dupont de Nemours (Hamburg, Germany), technical grade isoproturon (98% purity) by Bayer Crop Science (Monheim, Germany), and technical grade bentazone (98,4% purity) by BASF (Limburgerhof, Germany). Methanol, acetonitrile, and water were of A.R. grade (VWR, Leuven, Belgium).

4.3.1.2 Matrix substratum description

The organic substratums included in the columns are described in detail in Chapter 2 and were peat mix, garden waste compost, straw, sandy loam soil, dried cow manure, willow chopping and coco chips. The characteristics of these substratums are presented in Table 2.1

4.3.1.3 Column set-up

Column microcosms were packed in triplicate with five different mixtures of air-dried organic substratums and sandy loam soil as reported in Table 4.1. Column macrocosms were packed in duplicate with ten different mixes of air-dried organic substratums and sandy loam soil as reported in Table 4.2. For the column microcosms, substratum amounts were measured gravimetrically, manually mixed in a bucket for about 5-10 minutes to form homogeneous mixtures, and then packed into glass columns (Figure 4.1A). Compaction of the matrix was carried out by placing a weight of 5 *kg* on top of the matrix. The columns were 15 *cm* high with an inner diameter of 10 *cm*. The bottom of the columns was lined with a glass filter, so that seepage face conditions could develop there. The substratums in the column macrocosms (polyethylene barrels, height: 50 *cm*, inner diameter: 45 *cm*) were mixed with a concrete mixer to form homogeneous mixes, and then packed into plastic barrels till a height of 45cm (Figure 4.1B). A temperature USB data logger (EL-USB-LITE, Lascar, Switzerland) which records temperature every 30 *min* was positioned at a depth of about 22.5 *cm* in the matrix. The bottom of the barrel was filled with Taunus quartz covered with anti-root foil to prevent migration of small parts of the organic matrix. Batch sorption experiments were performed to asses sorption of the studied pesticides on the silicone tubes, Taunus quartz, and anti-root foil, but no significant sorption was observed (data not shown). A silicone tube was connected to a hole in the bottom of the barrel to collect effluents. The hole was covered with gauze to prevent blockage of the tube and to allow free drainage. All experiments were performed at room temperature.

Table 4.1: Composition of the substratum matrices(mix 1-5) as applied in the microcosm systems (g).

	MIX 1	MIX 2	MIX 3	MIX 4	MIX 5
Garden waste compost	/	159.93	/	/	/
Straw	27.33	28.43	27.27	12.97	13.00
Coco chips	/	/	/	52.43	52.57
Peat mix	105.20	/	87.03	104.77	83.87
Sandy loam soil	641.07	643.27	640.63	642.33	639.67
Cow manure	/	/	64.27	/	64.27

Table 4.2: Composition of the substratum matrices (mix 1-10)s as applied in the macrocosm systems (kg).

	MIX 1	MIX 2	MIX 3	MIX 4	MIX 5	MIX 6	MIX 7	MIX 8	MIX 9	MIX 10
Garden waste compost						8.17	14.71			6.54
Cow manure								3.23	1.29	3.23
Coco chips			2.57	5.13			10.5		5.13	2.05
Peat mix	5.36	9.44	5.25	9.44	5.25			4.17	8.45	4.28
Straw	1.41	1.35	0.68		0.68	1.36		1.36		0.68
Willow chopping					6.31					1.26
Sandy loam soil	30.24	6.04	30.27	6.04	30.23	30.15	6.04	30.18	6.04	6.04

A.

B.

Figure 4.1: Experimental set-up of the column A. micro- and B. macrocosms

4.3.1.4 Displacement experiments

Displacement experiments were conducted under unsaturated, steady-state flow conditions. Steady state water flow conditions were established prior to the application of the solute step input. A $CaCl_2$ solution (0.001*M* $CaCl_2$) was supplied to the column surface using PTFE (Polytetrafluoroethylene) tubes and a peristaltic pump (Type 205S/CA, Watson Marlow, Zwijnaarde, Belgium) delivering a constant Darcy flux of respectively 1.74 *cm* d^{-1} and 0.90 *cm* d^{-1} for the micro- and macrocosms. Droplets fell on a paper filter placed on the matrix substratums in order to provide a homogenous distribution of the solution. The top of each

microcosm column was covered with parafilm, while the macrocosms were covered with a plastic lid with a small opening for the inlet to avoid evaporation. It was assumed that steady-state conditions had been reached once the mass of the column remained constant in time. When steady-state conditions were achieved, pesticides were applied to the column, initially together with a bromide solution (0.1 *mM* Br^-). The pesticide solution pumped onto the column contained 0.001 *M* $CaCl_2$ and 10 *mg* L^{-1} linuron, isoproturon, metalaxyl and bentazone. While pesticides were added continuously as a step input, the bromide solution was applied as a pulse with a duration of respectively 18.3 *h* and 44.5 *h* for the micro- and macrocosms. The effluent was collected in a fraction collector at the bottom every 2-3 days (the effluent was the total amount of solution eluted during two consecutive sampling days) and both outflow volumes, pH and pesticide concentrations were measured. Bromide in the form of KBr was used as a non-reactive tracer in the column experiments to determine physical transport parameters. Bromide concentrations were determined using ion chromatography (Dionex ICS 2000), containing an AS15 column and KOH elluent. Bromide detection was performed by conductivity with a detection limit of 0.001 *mM*. In all cases, the experiments lasted for about 100 - 150 *d* until the effluent concentrations of most pesticides reached a constant value. Pesticide effluent concentrations were determined as described in chapter 3.

4.3.2 Transport models

The HYDRUS-1D model for simulating one-dimensional water flow and transport of solutes in soils was used to describe the transport of pesticides in the column[105]. It was assumed that experimental breakthrough curves (BTCs) could be described using the classical convection-dispersion transport model that neglects both physical and chemical non-equilibrium[135]. Chemical non-equilibrium[135] was not considered since the sorption process for the pesticide-substratum combinations was sufficiently fast under the applied low flow rates to establish local equilibrium (*Cfr*. 3.3.4.1).

Assuming that decay is only active in the liquid phase, and since a non-linear relationship between liquid and solid phase concentrations can be described using the Freundlich adsorption isotherm: $C_s = K_f C_l^n$, where C_l [*mg* L^{-1}] and C_s [*mg* kg^{-1}] are concentrations in the liquid and solid phases, respectively, and K_f and *n* are Freundlich parameters, the transport of a reactive solute for steady-state water flow conditions can be written as:

$$\frac{\partial C_l}{\partial t} = D\frac{\partial^2 C_l}{\partial z^2} - v\frac{\partial C_l}{\partial z} - \frac{\rho_b}{\theta}\frac{\partial C_s}{\partial t} - \mu_l C_l \qquad (4.1)$$

where *D* is the dispersion coefficient [$cm^2\ h^{-1}$], *v* is the pore water velocity [*cm* h^{-1}], $v = q/\theta$, in which *q* is the Darcian water flux [*cm* h^{-1}] and θ is the volumetric water content [$cm^3_{water}\, cm^{-3}_{pores}$], ρ_b is the bulk density [*g* mL^{-1}], μ_l is the first-order degradation constant for the solute in the liquid phase [h^{-1}], and *t* [*h*] and *z* [cm] are the temporal and spatial coordinates, respectively. The change in the sorbed concentration can be written as follows:

$$\frac{\rho_b}{\theta}\frac{\partial C_s}{\partial t}=\frac{\rho_b}{\theta}\frac{dC_s}{dC_l}\frac{\partial C_l}{\partial t}=\frac{\rho_b}{\theta}K_f n C_l^{n-1}\frac{\partial C_l}{\partial t} \tag{4.2}$$

Finally, incorporating (4.2) into (4.1) leads to:

$$R\frac{\partial C_l}{\partial t}=D\frac{\partial^2 C_l}{\partial z^2}-v\frac{\partial C_l}{\partial z}-\mu_l C_l \tag{4.3}$$

where *R* is the retardation factor (-):

$$R=1+\frac{\rho_b}{\theta}K_f n C_l^{n-1} \tag{4.4}$$

In the model described above (referred to below as the convection-dispersion equation (CDE) model), pesticide degradation is assumed to be a first-order process depending only on pesticide concentration. However, this is in contrast with an adaptation period or lag phase (a start-up period, during which degradation is not detected) commonly observed in laboratory mineralization experiments[136]. The occurrence of a lag phase might be attributed to enzyme induction or to a low level of pesticide degrading populations. Therefore, we incorporated the simplified version of the Monod kinetics[137] to describe BTCs where the adaptation period was clearly present (*e.g.*, metalaxyl BTCs) into HYDRUS-1D. In such a model, bacterial growth can be described using the following Monod kinetics:

$$\frac{dX}{dt}=\left(\mu_m\frac{C_l}{K_s+C_l}\right)X-k_{decay}X \tag{4.5}$$

where *X* is the pesticide-degrading biomass concentration [$mg_b\ L^{-1}$], C_l is the liquid pesticide concentration [$mg_p\ L^{-1}$], μ_m is the mass growth rate [h^{-1}], K_s is the half saturation constant [$mg_p\ L^{-1}$], and k_{decay} is the decay rate [h^{-1}] (subscripts *b*, *p* and *w* refer to biomass, pesticide and water, respectively). The bacterial Monod growth function can be related to the pesticide consumption utilizing a yield coefficient, *Y*, defined as a mass ratio of the organisms formed per pesticide utilized [$mg_b\ mg_p^{-1}$]. The local change in pesticide concentration when neglecting the effects of transport can then be expressed as follows:

$$\frac{dC_l}{dt}=-\frac{1}{Y}\left(\mu_m\frac{C_l}{K_s+C_l}\right)X \tag{4.6}$$

If we assume that the pesticide concentration (C_l) is significantly lower than the half saturation constant ($C_l << K_s$), then equations (4.5) and (4.6) simplify as follows:

$$\frac{dX}{dt}=\left(\frac{\mu_m}{K_s}C_l\right)X-k_{decay}X=\left(\mu_m^* C_l\right)X-k_{decay}X \tag{4.7}$$

$$\frac{dC_l}{dt} = -\left(\frac{\mu_m}{YK_s} X\right) C_l = -(\mu^* X) C_l \tag{4.8}$$

where μ_m^* is the modified mass growth rate [*L* $mg_p^{-1}h^{-1}$], and μ^* has units of [*L* $mg_b^{-1}h^{-1}$]. Incorporating Eq. (4.8) into the one-dimensional transport equation (4.3), results in:

$$R\frac{\partial C_l}{\partial t} = D\frac{\partial^2 C_l}{\partial z^2} - v\frac{\partial C_l}{\partial z} - (\mu^* X) C_l \tag{4.9}$$

These equations (referred to below as the Monod model) have been implemented in HYDRUS-1D. The fitting parameters for this model are μ^*, μ_m^* and k_{decay}.

4.3.3 Batch degradation experiments

At the end of the macrocosms displacement experiment, the matrix was removed from the barrels and divided into three parts, *i.e.* lower (0-15 *cm*), middle (15-30 *cm*) and upper (30-45 *cm*) part. 0.500 ± 0.001 *g* of matrix of the upper and lower layer of mix 3, 4, 9, and 10 and of fresh material with the same composition as mix 9 were transferred into an autoclaved Erlenmeyer. Experiments were carried out in triplicate for each mix and layer. 50 *mL* of MMO (mineral medium without any carbon source but with nitrogen containing salts) medium containing 20 *mg* L^{-1} of linuron, isoproturon, bentazone, and metalaxyl, which was prepared as described by Dejonghe *et al.*[138], was added to the Erlenmeyer and incubated on a shaker at 150 *rpm* at room temperature. A sterile control, to check for abiotic losses, was prepared for each mix-layer combination by adding chloroform (8% v/v) to the MMO solution. Every 2-3 days 800 *µL* of the solution was sampled and filtered with a syringe filter with a PVDF membrane with a pore size of 0.22 *µm* (Carl Roth, Karlsruhe-Rheinhafen, Germany). The aliquot was analysed with HPLC-DAD (see 3.3.3).

4.3.4 Pesticide extraction from the matrix

The three fractions of the organic matrix from the macrocosms (0-15 *cm*, 15-30 *cm*, and 30-45 *cm)* were analyzed to quantify the residual pesticides. Extraction of the pesticides was carried out on 50.000 ± 0.001 *g* organic matrix of which the dry matter content was determined gravimetrically after drying at 105°C during 24 *h*. 200 *mL* of methanol was added to the organic matrix and shaken during 1 *h* at 150 *rpm*. The liquid phase was separated from the solid phase with a Buchner filter. These steps were repeated three times after which the liquid phases were collected and evaporated with a rotavapor. The pesticides were re-dissolved in 10 *mL* 90:10 (v/v) methanol – 0.1% H_3PO_4 water solution and analysed with HPLC-DAD (see 3.3.3).

4.4 Results and discussion

Physical transport was first evaluated by analyzing the bromide BTCs. The volumetric water content, θ, and the longitudinal dispersivity, λ ($D=\lambda \cdot v$), were fitted to the observed Br^- BTCs. Once the physical transport was fully characterized using bromide BTCs, chemical processes for isoproturon, metalaxyl, linuron, and bentazone were analyzed by inverting the pesticide BTCs with the Levenberg-Marquardt algorithm in HYDRUS-1D, using the physical transport parameters estimated for bromide transport. In all inversions of the pesticide BTCs, the soil water content, θ, the water flux, q, the bulk density, ρ, and the dispersivity, λ, were fixed. Estimated reaction parameters included the Freundlich parameters K_f and n, and the liquid degradation constant μ_l with a first-order degradation or the Monod kinetic parameters μ^*, μ_m^* and k_{decay} when delayed degradation occurred. The pH of the effluent of the macrocosms was fairly constant and varied between 6.65 and 8.67. The temperature of the organic mix in the macrocosms varied between 13.3°*C* and 21.2°*C* and followed the fluctuations in room temperature.

4.4.1 Transport in microcosms

4.4.1.1 Bromide breakthrough curves (BTCs)

BTCs of the microcosms are plotted in Figure 4.2 as Br^- concentrations versus the number of pore volumes (*PV*) eluted (one pore volume represents the time needed for the volume of water that fills the voids of the column to be eluted: $PV=qt/(\theta L)$). The HYDRUS-1D model fitted the experimental data well with R^2 varying between 0.70-0.92 (Table 4.3). However, the fit to the Br^- BTCs of mixture 5A and 5B is poorer due to the presence of a plateau in the experimental data. High values before and after the peak could be caused by analytical errors. The experimental BTCs did not show any significant asymmetry or long-tailing that would indicate physical non-equilibrium. The mass balance of the amount of Br^- entering and leaving the column showed an overall average mass recovery of 96%, and therefore it could be concluded that physical equilibrium prevailed. Fitted transport parameters are presented in Table 4.3, together with bulk densities of the column microcosm. High water contents (in comparison to soils), likely caused by the high water sorbing capacity of organic substratums such as peat mix and coco chips, were found for all columns. The dispersivity length was quite similar for all mixtures, with a slightly higher dispersion for mixtures 3 and 4. Only slight differences existed in transport parameters between replicated columns. These differences could be explained by the spatial heterogeneity of the structure of the organic substratums and packing of the columns.

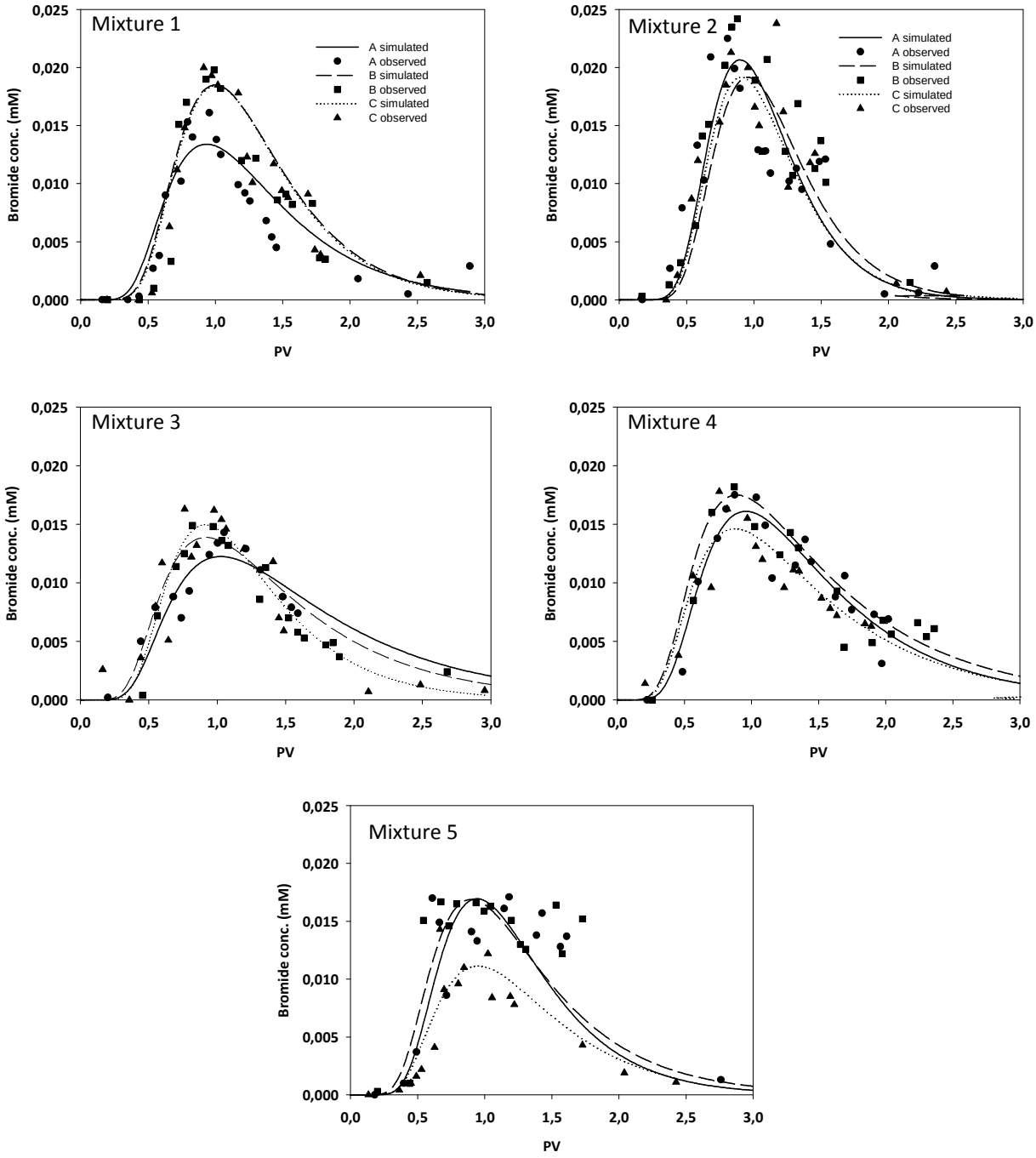

Figure 4.2: Observed and simulated BTCs of Br^- in 5 experimental microcosms set-ups (replicates A, B, and C) containing different mixture substratums. Symbols represent the observed data, full, dashed and dotted lines the model fits. Absolute bromide concentrations (mM) are plotted against the number of pore volumes (PV).

Table 4.3: Estimated physical transport parameters for Br^- in the 5 microcosms using the CDE transport model (± 95% confidence interval).

MIX	θ ($cm^3_{water}\,cm^{-3}_{pores}$)	λ (*cm*)	R^2	ρ ($g\,mL^{-1}$)
1A	0.68 ± 0.03	2.20 ± 0.34	0.86	0.66
1B	0.53 ± 0.02	1.61 ± 0.28	0.87	0.66
1C	0.54 ± 0.02	1.56 ± 0.20	0.91	0.66
2A	0.51 ± 0.02	1.88 ± 0.31	0.81	0.70
2B	0.52 ± 0.02	1.14 ± 1.14	0.85	0.70
2C	0.55 ± 0.02	1.12 ± 0.23	0.78	0.71
3A	0.69 ± 0.03	3.04 ± 0.51	0.84	0.69
3B	0.57 ± 0.04	3.33 ± 0.76	0.91	0.70
3C	0.63 ± 0.02	1.97 ± 0.29	0.87	0.70
4A	0.53 ± 0.05	2.99 ± 0.36	0.90	0.69
4B	0.47 ± 0.02	4.28 ± 0.72	0.87	0.69
4C	0.57 ± 0.02	3.82 ± 0.49	0.87	0.69
5A	0.57 ± 0.04	1.86 ± 0.56	0.70	0.73
5B	0.53 ± 0.03	2.50 ± 0.55	0.75	0.72
5C	0.83 ± 0.04	2.13 ± 0.42	0.80	0.72

4.4.1.2 Isoproturon BTCs

Isoproturon BTCs are shown in Figure 4.3 in terms of relative concentrations (*i.e.*, measured concentrations relative to the inlet concentration) versus the number of pore volumes (*PV*) eluted. The fitted solute reaction parameters are presented in Table 4.4. The CDE model described the isoproturon BTCs well, as reflected in high values of the determination coefficient R^2 (0.95 – 1.00) (Table 4.4).

The BTCs shifted significantly more to the right than the BTCs of bromide (Figure 4.2), which indicates that the pesticide is strongly sorbed to the organic substratums, which is also reflected by the retardation coefficient *R* (Table 4.4). However, *R* (which is calculated using the initial concentration as input concentration), is slightly overestimated if degradation occurs. Mixtures 1 and 2 retained isoproturon the most, with an average *R* of 12 and 15, respectively. Mixtures 3, 4 and 5 retained isoproturon in a similar way, with an average *R* of 7, 8 and 7, respectively. This indicates that the classical biomix composition as in mixture 1 and 2 containing peat mix or garden waste compost retains isoproturon the most. Decreasing the amount of peat or straw appears to decrease the retention of this pesticide. The value of the Freundlich exponent n_{column} is an indicator of the curvature of the sorption isotherm. Most sorption isotherms are of the S-type ($n > 1$), which indicates a strong competition by water molecules for adsorption sites at low pesticide concentrations [108]. However, the sorption of isoproturon on mixtures 1, 3 and, 4 was almost linear. Also note that the uncertainty of this parameter was relatively low (*i.e.*, narrow confidence intervals, see Table 4.4). However, this was true only when sorption was almost linear. Notice that when sorption was nonlinear, the uncertainty increased significantly (Table 4.54). A comparison of the Freundlich parameters obtained using column transport experiments with

those obtained in batch experiments in Chapter 3 (Table 4.43.3) showed that $K_{f,\ column}$ values were about 2.4-6.9 times smaller for the column experiments.

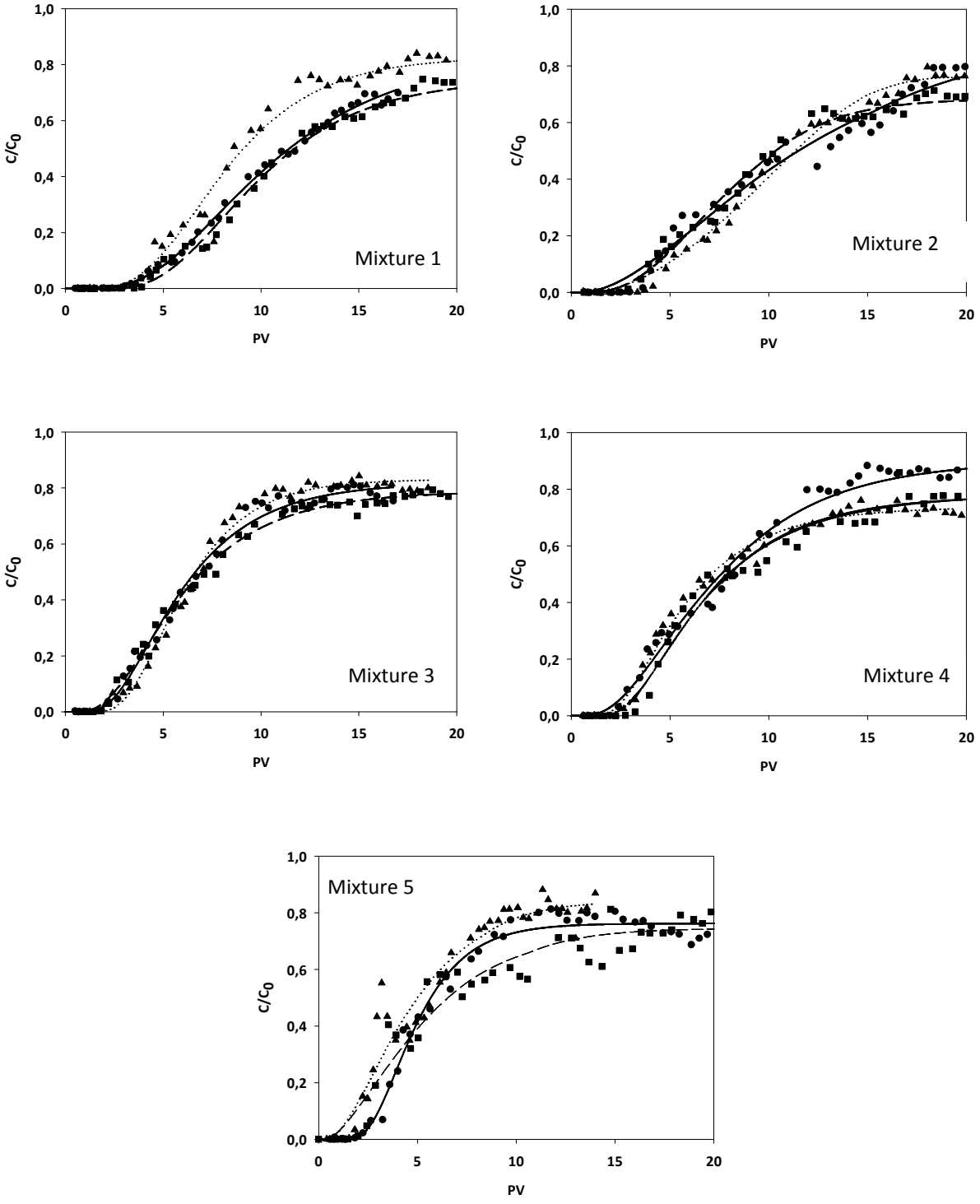

Figure 4.3: Observed and simulated BTCs of isoproturon in 5 experimental column set-ups (replicates A, B, and C) containing different mixture substratums. Symbols represent the observed data, full, dashed and dotted lines the model fits. Relative concentrations are plotted against the number of pore volumes (PV).

This means that isoproturon is more strongly sorbed by substratums in batch experiments, and that using coefficients based on such studies in transport models would overestimate sorption. This was reported previously by Meyer-Windel *et al.*[139] for atrazine and isoproturon, and Kookana *et al.*[140] for fenamiphos, linuron and simazine. In the present study inverse modeling of the BTCs was performed with the Freundlich parameters obtained in batch experiments. Nonetheless optimizations with chemical equilibrium and non-equilibrium assumptions did not improve the fit of the BTC. Batch sorption experiments are not always representative of the sorption process occurring in column studies or in the field, as the solid/liquid ratio is much higher in percolate column experiments than in batch experiments. There could also be an increase in surface area in batch experiments resulting from the disaggregation of clusters during shaking. Moreover, the pesticide-substratum solution is continuously shaken, which improves contact between pesticide and substratum, which could enhance sorption[141]. However, there also exists an alternative opinion that is supported by a number of observations that show that results from batch experiments can be used to describe the transport behavior of pesticides in soil columns and outdoor if essential processes such as non-equilibrium sorption are considered (*e.g.*[142-146]).

Since the inlet concentration of isoproturon is not reached at the outlet, degradation probably occurs inside the columns, as is also reflected by the positive values of the first-order degradation rate μ_l (Table 4.4). Differences in degradation potential between the mixtures were minimal, all degradation constants (μ_l) were of the same order. Thus, the composition of the matrix appears to have little influence on degradation of isoproturon. As degradation is only considered in the liquid phase, half-life values ($t_{1/2}$) need to be calculated from μ_l/R instead of from μ_l for sorbing chemicals. Half-life ($t_{1/2}$) values of isoproturon were calculated and presented in Table 4.4. According to the $t_{1/2}$ value, degradation was slightly higher in mixture 3, 4 and 5, while it was lower in mixture 1 and 2. The values are much higher than the reported values in literature ($t_{1/2,\text{soil}}$= 6-20 *d*)[85,147]. Sorption to organic substratums can decrease the bioavailability and therefore decrease biodegradation.

Table 4.4: Solute reaction parameters fitted to isoproturon BTCs (± 95% confidence interval).

MIX	$K_{f,\ column}$ [$L\ kg^{-1}$]	n_{column}	$K_{f,\ batch}$ [$L\ kg^{-1}$]	n_{batch}	μ_l (x 10^{-3}) [h^{-1}]	R^2	R	$t_{1/2}$ (*d*)
1A	9.30 ± 0.39	1.12 ± 0.02			1.24 ± 0.18	1.00	14.19	331
1B	7.81 ± 0.44	1.03 ± 0.04	18.28	0.89	2.74 ± 0.18	0.99	11.84	125
1C	5.60 ± 0.63	1.09 ± 0.05			1.75 ± 0.38	0.98	10.18	168
2A	3.27 ± 0.40	1.43 ± 0.07			1.15 ± 0.17	0.98	18.06	452
2B	3.47 ± 0.29	1.26 ± 0.04	11.72	0.81	3.64 ± 0.12	0.99	11.60	92
2C	2.11 ± 0.10	1.56 ± 0.02			2.43 ± 0.12	0.99	16.50	196
3A	5.93 ± 0.31	1.01 ± 0.05			1.45 ± 0.09	0.99	7.09	141
3B	4.32 ± 0.38	1.09 ± 0.05	17.15	0.90	2.10 ± 0.09	0.99	8.10	112
3C	5.65 ± 0.59	0.95 ± 0.06			1.46 ± 0.16	0.99	6.40	126
4A	3.98 ± 0.40	1.17 ± 0.05			0.98 ± 0.04	0.99	9.87	292
4B	6.19 ± 0.07	0.90 ± 0.01	18.37	0.93	2.72 ± 0.12	0.98	7.52	80
4C	5.89 ± 0.49	0.92 ± 0.05			2.76 ± 0.10	0.99	6.53	68
5A	4.07 ± 0.43	0.94 ± 0.05			2.37 ± 0.28	0.99	5.26	64
5B	1.44 ± 0.43	1.47 ± 0.16	17.25	0.93	2.78 ± 0.19	0.95	9.53	99
5C	1.98 ± 0.45	1.41 ± 0.12			1.03 ± 0.00	0.95	7.30	205

4.4.1.3 Bentazone BTCs

Experimental bentazone BTCs were also analyzed using the CDE model (Figure 4.4,Table 4.5). The determination coefficient R^2 ranged from 0.51 to 0.91. The arrival of bentazone, which is a very mobile pesticide, was very similar to that of the inert tracer bromide, showing that this pesticide is hardly retained in the organic substratums.

Since the value of the Freundlich exponent n_{column} has been proven to be highly uncertain (high standard deviations in Table 4.5), the retardation factor was calculated assuming linear adsorption (n=1). Reported retardation factors are therefore underestimated because the n exponent is larger than 1. In general, the retardation factors R were close to 1, indicating that hardly any retention occurred for all mixtures, and confirming Gaston and Locke's[148] results with columns packed with Dundee silty clay loam soil. The fitted n_{column} values for most mixtures were relatively high, as compared to n values for bentazone reported in the literature, which ranged from 0.88 to 1.061 for Mississippi and coastal plain soils[149,150]. However, as discussed above, these values are highly uncertain. The n_{column} values are determined by the changing slope of the part of the BTC curve where C/C_0 increases from 0 to equilibrium conditions. Since bentazone breakthrough occurs very fast, the number of data points in this part of the curve is limited. This results in a poor estimation of the n value. Calculated $K_{f,\ column}$ values for bentazone are much smaller than those obtained from the batch experiments (Table 4.5), as was found for isoproturon. However, it should be noted that $K_{f,\ column}$ and n_{column} values were very uncertain as a result of the limited number of data points in the early part of BTCs. Correlations between the two sorption parameters were as high as 0.99. Note that the uncertainty of bentazone sorption parameters and their correlation is reflected in the relatively large confidence intervals. Because of this uncertainty, it is also difficult to compare various mixtures with respect to their bentazone retention capacity.

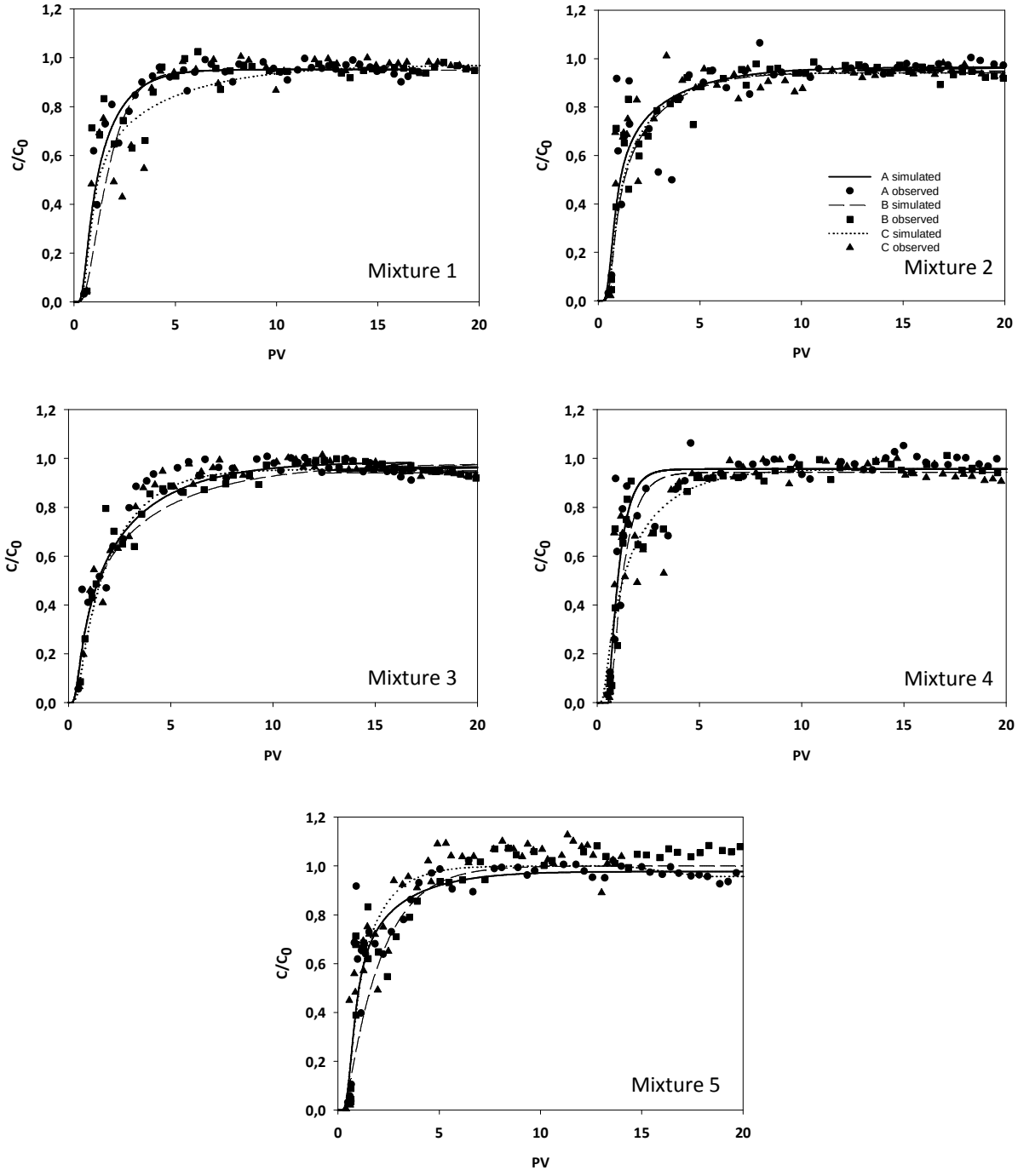

Figure 4.4: Observed and simulated BTCs of bentazone in 5 experimental column set-ups (replicates A, B, and C) containing different mixture substratums. Symbols represent the observed data, full, dashed and dotted lines the model fits. Relative concentrations are plotted against the number of pore volumes (PV).

Degradation of bentazone was almost negligible. Outflow bentazone concentrations quickly approached the input concentration, which indicated very limited degradation. This is also confirmed by the values of the half-life of bentazone which were very high (> 7828 days). This is in contrast to the literature, where bentazone was described as a very low persistent molecule with a half-life in soil of less than two weeks[151]. However, these studies were not

performed in column experiments but in batch experiments. Low degradation of bentazone in our column studies could be expected because of the very short residence time in the columns, which decreases the probability for biodegradation. Finally, as for isoproturon, composition of the matrix was of little importance for degradation because small differences in degradation efficiency between the organic mixtures were present and values were of the same order of magnitude.

Table 4.5: Solute reaction parameters fitted to bentazone BTCs (± 95% confidence interval)

MIX	$K_{f,\ column}$ [$L\ kg^{-1}$]	n_{column}	$K_{f,\ batch}$ [$L\ kg^{-1}$]	n_{batch}	μ_l (x 10^{-3}) [h^{-1}]	R^2	R	$t_{1/2}$ (*d*)
1A	$1.35\cdot10^{-3} \pm 2.95\cdot10^{-3}$	3.62 ± 0.96			3.51 ± 1.02	0.92	1.00	14032
1B	$1.41\cdot10^{-1} \pm 1.06\cdot10^{-1}$	1.66 ± 0.31	3.42	0.81	4.84 ± 0.54	0.90	1.29	10516
1C	$1.75\cdot10^{-5} \pm 6.03\cdot10^{-2}$	5.86 ± 1.57			2.79 ± 0.00	0.71	1.00	18932
2A	$5.45\cdot10^{-6} \pm 1.26\cdot10^{-5}$	6.12 ± 0.99			3.45 ± 1.28	0.88	1.00	14427
2B	$1.42\cdot10^{-4} \pm 1.83\cdot10^{-4}$	4.74 ± 0.57	2.93	0.80	5.67 ± 1.11	0.92	1.00	8398
2C	$9.07\cdot10^{-2} \pm 1.13\cdot10^{+1}$	0.00 ± 4.55			6.21 ± 1.07	0.81	1.00	7828
3A	$6.11\cdot10^{-3} \pm 1.48\cdot10^{-2}$	3.39 ± 1.12			1.02 ± 0.54	0.70	1.02	55619
3B	$2.70\cdot10^{-3} \pm 7.03\cdot10^{-3}$	3.79 ± 1.22	3.40	0.81	1.61 ± 1.07	0.69	1.01	28849
3C	$1.54\cdot10^{-2} \pm 4.10\cdot10^{-2}$	2.84 ± 1.15			3.55 ± 0.91	0.53	1.05	14028
4A	1.00 ± 1.03	0.12 ± 0.26			4.10 ± 1.25	0.82	1.16	49892
4B	1.31 ± 1.34	0.33 ± 0.55	4.10	0.81	6.12 ± 2.54	0.51	1.64	31518
4C	$4.75\cdot10^{-3} \pm 8.59\cdot10^{-3}$	3.23 ± 0.82			3.76 ± 1.62	0.85	1.02	51749
5A	$3.12\cdot10^{-6} \pm 9.91\cdot10^{-6}$	6.28 ± 1.39			1.96 ± 1.71	0.84	1.00	24249
5B	$5.84\cdot10^{-2} \pm 5.75\cdot10^{-2}$	2.15 ± 0.42	4.08	0.81	0	0.91	1.17	/
5C	$2.92\cdot10^{-4} \pm 1.04\cdot10^{-3}$	4.25 ± 1.51			0	0.87	1.00	/

[a] Retardation coefficient was calculated assuming linear sorption (n=1).

4.4.1.4 Metalaxyl BTCs

Transport of metalaxyl (both observed, as well as simulated using the CDE model) through the experimental columns is shown in Figure 4.5. Collected experimental metalaxyl BTCs for mixtures 2, 3, and 5 have a very different pattern than those obtained for isoproturon and bentazone. Although a step input was applied, the BTCs for mixtures 2, 3, and 5 showed a pattern that is characteristic for a pulse input. Effluent concentrations increased up to a certain value, and then began to decrease. A first-order degradation process could not be used to describe the observed effluent metalaxyl concentrations in these mixtures. It was hypothesized that these BTCs could be described using a Monod-type degradation model (eq. (4.7) and (4.9)) that considers bacterial growth and decay.

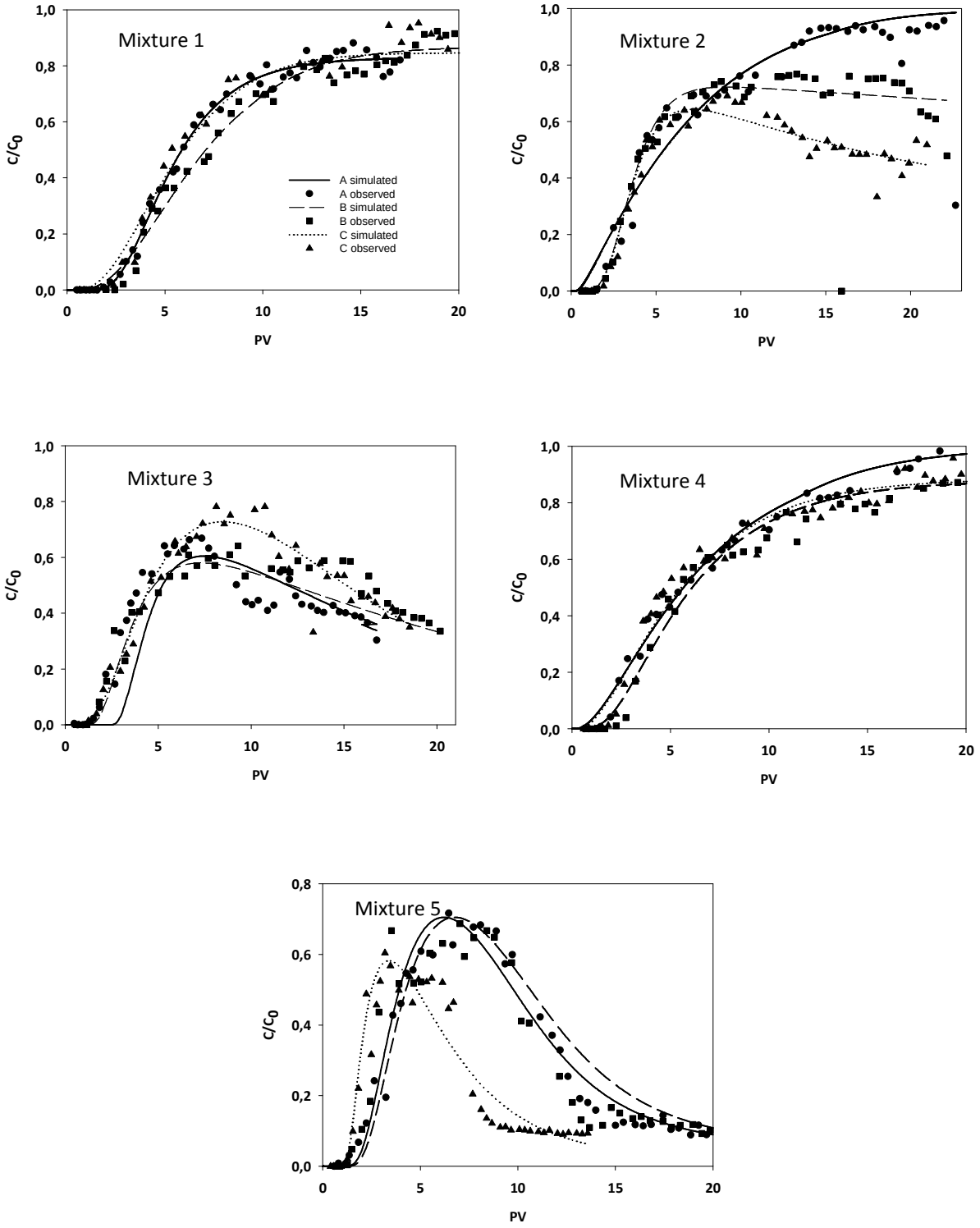

Figure 4.5: Observed and simulated BTCs of metalaxyl in 5 experimental column set-ups (replicates A, B, and C) containing different mixture substratums. Symbols represent the observed data, full, dashed and dotted lines the model fits. Relative concentrations are plotted against the number of pore volumes (PV).

The classical CDE model (4.3) was first used to fit the first part of the BTCs up to the maximum effluent concentration for mixtures 2, 3, and 5, in order to obtain the Freundlich parameters of this initial part of the BTCs. Although we realize that the Monod-type degradation is involved already during this first part of the BTC, its effects are relatively limited, and in this way we can obtain initial estimates of these reaction parameters. The determination coefficient R^2 for the CDE model ranged from 0.64 to 0.99 (Table 4.6). Since the retardation coefficient R for metalaxyl is higher than one, retention of this pesticide into the organic matrix occurs (Table 4.6). Retention of metalaxyl (average R = 8.51) is similar to that of isoproturon (average R = 7.97) in mixture 4, but is slightly lower in mixtures 1, 2, 3, and 5. This is in agreement with the more or less similar K_{oc} values of metalaxyl (K_{oc}= 47 $L\ kg^{-1}$) and isoproturon (K_{oc}= 36 $L\ kg^{-1}$). Retardation factors of 2.3-3.8 were observed by Fernandes *et al.*[152] for metalaxyl in agricultural soils but those had a lower organic matter content than the mixtures used in this study. This likely explains the higher retention found in our experiments.

The $K_{f,\ column}$ values of metalaxyl (Table 7) (average $K_{f,\ column}$ = 2.84 $L\ kg^{-1}$) were very similar to those of isoproturon (Table 4) (average $K_{f,\ column}$ = 4.74 $L\ kg^{-1}$). However, these values were again much lower than those obtained from batch experiments ($K_{f,\ batch}$ = 15.39 $L\ kg^{-1}$) (Table 4.6).

First-order degradation constants for metalaxyl in mixtures 1, 2a and 4 are presented in Table 4.6. The obtained values were of the same magnitude as those recorded for isoproturon, and close to those reported by Kookana *et al.*[140]. The $t_{1/2}$ values are very high for mix 2A and 4A because the effluent concentration in these mixtures reached the inlet concentration. Nonetheless, the overall $t_{1/2}$ value in the organic mixtures is similar or slightly lower to the values described in literature ($t_{1/2}$ > 200 days)[85]. Evidence of delayed degradation can be seen in mixtures 2, 3, and 5 (Figure 4.5). Two out of three replicated BTCs obtained with mixture 2 (B and C) showed a slight decrease of pesticide concentrations after reaching a maximum value. The decrease of metalaxyl concentrations, compared to the maximum value, was about 20% for replicate B and 31% for replicate C. However, a much more pronounced decrease in effluent concentrations was observed in mixtures 3 and 5. The decrease of effluent concentrations in mixture 3 was between 45 and 50% for different replicates, and in mixture 5 it was about 85%. The presence of cow manure is the only difference in the composition of mixtures 3 and 5, compared to mixture 1, 2, and 4. Cow manure represents a source of nitrogen, which could stimulate the growth of metalaxyl degrading bacteria. Although no specific results have been reported in the literature on the influence of manure on metalaxyl degradation, the effects of manure on degradation of other pesticides are well documented. For example, atrazine, metolachlor, and trifluralin degradation improved in the presence of manure[153], dealkylation reactions of *s*-triazines and phenylureas were also enhanced by manure[154]. Finally, Dolaptsoglou *et al.*[155], found that the incorporation of poultry manure accelerated the degradation of terbuthylazine.

Table 4.6: Predicted solute parameters for metalaxyl (± 95% confidence interval)

MIX	$K_{f,\ column}$ [$L\ kg^{-1}$]	n_{column}	$K_{f,\ batch}$ [$L\ kg^{-1}$]	n_{batch}	μ_l (x 10^{-3}) [h^{-1}]	R^2	R	$t_{1/2}$ (d)
1A	5.83 ± 0.48	0.95 ± 0.04			1.36 ± 0.08	0.99	5.75	122
1B	2.86 ± 0.33	1.26 ± 0.06	17.54	0.85	1.31 ± 0.14	0.98	9.27	204
1C	2.22 ± 0.59	1.28 ± 0.13			1.53 ± 0.26	0.94	7.60	144
2A	0.69 ± 0.25	1.78 ± 0.16			0.01 ± 0.09	0.90	11.10	22607
2B	1.88 ± 0.40	1.08 ± 0.10	9.46	0.89	/	0.96	4.27	/
2C	1.94 ± 0.32	1.07 ± 0.08			/	0.97	4.19	/
3A	4.58 ± 0.47	0.77 ± 0.06			/	0.92	3.09	/
3B	2.68 ± 0.55	0.94 ± 0.07	15.17	0.87	/	0.96	3.68	/
3C	2.95± 0.30	1.00 ± 0.07			/	0.92	4.29	/
4A	1.62 ± 0.36	1.47 ± 0.10			0	0.96	10.15	1006514
4B	3.74 ± 1.09	1.04 ± 0.14	18.58	0.85	1.36 ± 0.32	0.96	7.25	154
4C	2.39 ± 0.46	1.28 ± 0.10			1.06 ± 0.21	0.97	8.12	220
5A	3.11± 0.61	0.89 ± 0.11			/	0.64	3.72	/
5B	2.77 ± 0.57	0.76 ± 0.12	16.20	0.87	/	0.68	2.70	/
5C	3.22 ± 0.26	0.67 ± 0.05			/	0.93	1.87	/

The Monod kinetic parameters determined by fitting the entire experimental data curves are presented in Table 4.7. Determination coefficients of the fit using the CDE model (4.9) with the Monod kinetics (4.7) ranged between 0.86 and 0.96, indicating that the model described the experimental data very well. Since reaction coefficients of equation (4.7) are the same whether (4.7) was formulated in absolute or relative biomass concentrations, and the biomass concentration (X) in equation (4.8) is divided by the yield (Y), the actual absolute values of biomass can not be determined from the pesticide (C_l) breakthrough curve alone. To determine the biomass, at least one measured value of the biomass concentration is needed (*e.g.*, initial, final, or any other). This is because the biomass concentration and yield are fully correlated. Higher values of biomass concentrations and the correspondingly lower yield will produce identical results. For that reason, we carried out simulations in relative biomass concentrations with the initial value being equal to one. This results in the estimation of the modified mass growth rates which do not have any physical meaning and are only considered as fitting parameters. The obtained values are presented in Table 4.7. Thus the model should be interpreted as a conceptual model and not as a physical model. Nonetheless, it could be concluded that the Monod model described the effluent concentration of metalaxyl well, which indicates that metalaxyl dissipation is related to the growth of the biomass. Biomass growth might occur due to the presence of the organic substratum, which might be a suitable substrate for the biomass due to its high carbon content or might be enhanced by the presence of pesticides, which can serve as an additional carbon source. This growth of biomass might induce metalaxyl degradation or might increase metalaxyl sorption to the surface of the biofilm. However, as sorption does not occur for other pesticides (e.g. isoproturon which has similar sorptive behavior than metalaxyl), it is hypothesized that the application of metalaxyl selectively stimulated the growth of a metalaxyl degrading community.

Table 4.7: Monod kinetic parameters and the yield coefficient for the degradation of metalaxyl (± 95% confidence interval)

MIX	μ^* (x 10^{-7}) [$L\ mg_p^{-1}\ h^{-1}$]	μ_m^* (x 10^{-4}) [$L\ mg_b^{-1}\ h^{-1}$]	k_{decay} [h^{-1}]	R^2
2B	9.15 ± 14.02	-0.18 ± 8.21	$3.15 \cdot 10^{-6} \pm 7.01 \cdot 10^{-3}$	0.96
2C	13.43 ± 2.92	-2.11 ± 1.93	$1.08 \cdot 10^{-3} \pm 1.37 \cdot 10^{-3}$	0.96
3A	9.99 ± 0.89	-5.27 ± 1.09	$2.57 \cdot 10^{-3} \pm 6.22 \cdot 10^{-4}$	0.96
3B	10.95 ± 3.96	-0.98 ± 3.23	$2.30 \cdot 10^{-5} \pm 2.01 \cdot 10^{-3}$	0.86
3C	4.62 ± 0.95	-2.42 ± 1.41	$6.26 \cdot 10^{-4} \pm 1.06 \cdot 10^{-3}$	0.98
5A	3.38 ± 0.65	-3.95 ± 0.99	$5.27 \cdot 10^{-4} \pm 4.88 \cdot 10^{-4}$	0.93
5B	4.43 ± 0.70	-2.69 ± 0.54	$4.90 \cdot 10^{-8} \pm 2.14 \cdot 10^{-4}$	0.91
5C	1.99 ± 0.25	-2.51 ± 0.93	$1.12 \cdot 10^{-7} \pm 4.30 \cdot 10^{-4}$	0.90

4.4.1.5 Linuron BTCs

Linuron is a pesticide with a higher K_{oc} value than isoproturon, metalaxyl, and bentazone. Thus it is classified as immobile. Its low mobility in soil was previously reported by Guzzella *et al.*[156], Hance[157] and MacNamara and Toth[158]. No breakthrough of this pesticide was observed in 4 mixtures, even after about 20 PV. Breakthrough occurred only in case of mixture 4 (Figure 4.6). Replicate A did not show clear evidence of delayed dissipation, which was apparent for replicates B and C. However, from the final point of the BTC there was an indication that linuron effluent concentrations will also decrease for replicate A.

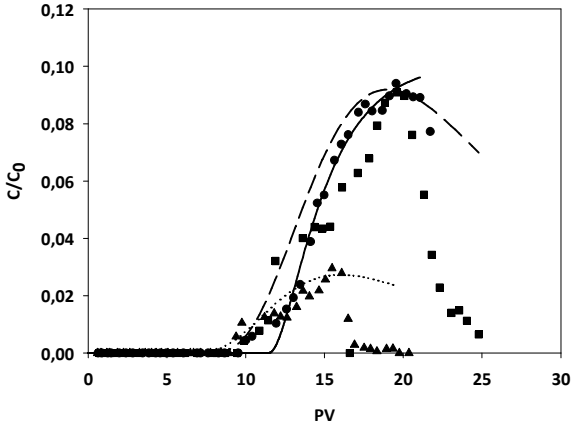

Figure 4.6: Observed and simulated BTC of linuron in columns containing mixture 4 (in triplicate: A, B, C) which consist of 25% sandy loam soil, 25% peat mix, 25% coco chips, 25% straw. Symbols are the observed data, full, dashed and dotted lines the model fits given by the convection-dispersion equation. Relative concentrations are presented against the number of pore volumes (PV).

As for metalaxyl, the CDE model was used for the first part (up to the maximum effluent concentration) of the BTCs to determine the Freundlich parameters (Table 4.8) (R^2 = 0.90-0.93). The $K_{f,column}$ constants were much higher than for the other pesticides, which was expected because of the late arrival and the high K_{oc} value of linuron. Again, $K_{f,column}$ values obtained from column experiments (average $K_{f,column}$ = 29.08 $L\ kg^{-1}$) were lower than those

calculated from the batch experiments (average $K_{f,batch}$ = 88.35 $L\ kg^{-1}$) (Table 4.8). The high retardation coefficient R confirms the high retention of linuron on the organic substratum. However, a lower retardation coefficient could be observed for replicate A due to the very low *n* value, which leads to a decrease in sorption with increasing concentration. First-order degradation constant of mix 4A is one order of magnitude higher than those for metalaxyl and isoproturon, likely explaining why no breakthrough occurred in the other mixtures (Table 4.8). A combination of the strong adsorption, and a correspondingly long resident time of linuron in the column, along with high degradation probably resulted into a complete degradation of linuron before it reached the bottom of the column. Linuron has previously been reported as a substrate for bacterial growth by Cullington and Walker[159] and by Widehem *et al.*[160].

Table 4.8: Predicted solute parameters of linuron (± 95% confidence interval)

MIX	$K_{f,\ column}$ [$L\ kg^{-1}$]	n_{column}	$K_{f,\ batch}$ [$L\ kg^{-1}$]	n_{batch}	μ_l (x 10^{-2}) [h^{-1}]	R^2	R	$t_{1/2}$ (d)
4A	29.52 ± 0.42	0.46 ± 0.03			2.98 ± 0.03	0.93	6.12	142
4B	27.85 ± 0.72	0.78 ± 0.07	88.35	0.75	/	0.91	20.15	/
4A	28.05 ± 2.18	0.83 ± 0.09			/	0.90	20.10	/

The BTCs of replicates B and C were fitted with the CDE model combined with the Monod kinetics. However, very low effluent concentrations and incomplete breakthrough resulted in a poor model fit, with R^2 ranging from 0.47 to 0.50. Monod kinetic parameters will not be further presented due to the their negligible significance.

4.4.2 Transport of pesticides and the inert tracer bromide in macrocosms

4.4.2.1 Bromide BTCs

BTCs of Br^- in the macrocosms are plotted in Figure 4.7. Due to the large amount of data and the good reproducibility, the average of two replications was presented in the graphs and tables. The HYDRUS-1D model fitted the experimental data well with R^2 varying between 0.76-0.99.

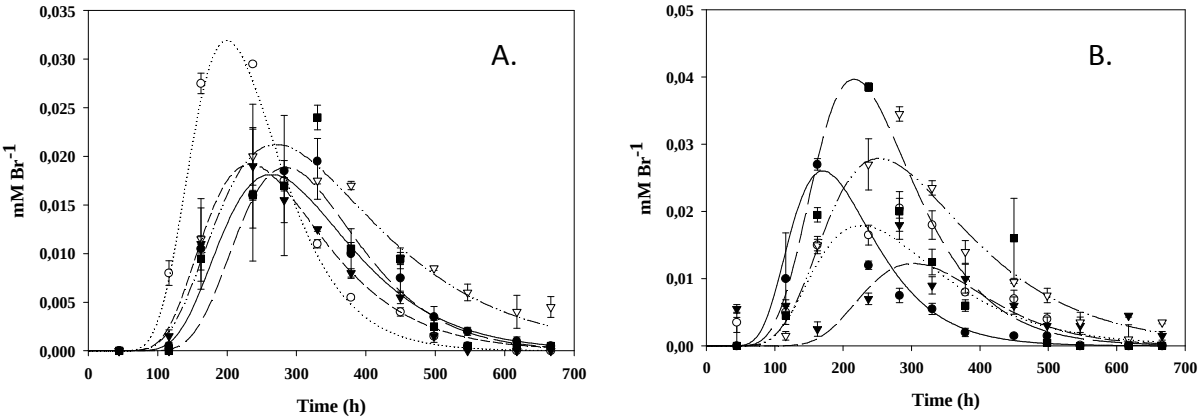

Figure 4.7: Observed and simulated BTCs of Br- in 10 experimental macrocosm set-ups containing different mixture substratums. Observed BTCs are presented with the following symbols in A: ● , mix 1; ○ , mix 2; ▼ , mix 3; ▽ , mix 4; ■ , mix 5. Simulated BTCs are presented as: ——— , mix 1; ····· , mix 2; — — , mix 3; —·— , mix 4; ——— , mix 5 and in B: ● , mix 6; ○ , mix 7; ▼ , mix 8; ▽ , mix 9; ■ , mix 10. Simulated BTCs are presented as: ——— , mix 6; ····· , mix 7; — — , mix 8; —·— , mix 9; ——— , mix 10. Absolute concentrations are plotted against time (h).

A high overall mass recovery of Br^- (94%) and the absence of significant asymmetry and tailing, indicates that physical equilibrium probably prevailed. Fitted transport parameters of two replications are presented in Table 4.9, together with bulk densities of the columns. The bulk densities of mix 1, 3, 5, 6, and 8 are higher than those of others due to the larger fraction of sandy loam soil. All mixes showed a similar water content. However, comparing the water content of mixes 1, 3, 6, and 8 with mixes (respectively, 1, 4, 2, and 3) with the same composition used in the microcosms (4.3.1.3), it is observed that the water content was much lower in the large-scale columns. This is attributed to the lower flux (0.90 $cm\ d^{-1}$) applied in the macrocosms compared to the microcosms (1.84 $cm\ d^{-1}$) and is in accordance with the results of Costa and Prunty[161]. The average dispersivity λ in the macrocosm system was about 1.5 times higher than this in the microcosms (resp. 3.59 and 2.37 *cm*) due to an increase in the displacement length (*i.e.* column length) (from 15 cm to 45 cm). This is a well established phenomenon described earlier by Wierenga and van Genuchten[162], Butters and Jury[163], Gelhar *et al.*[164], and Vanderborght and Vereecken[165]. An increase in disperisivity can also be attributed to an increase in system diameter[166]. In addition, dispersivity values are

correlated with pore-size distribution and magnitude of matrix structure heterogeneity. The high λ values observed in the macrocosm system, indicate a heterogeneous pore system or a long flow-path length[167]. Due to the large column volume, uneven packing probably caused heterogeneity in the matrix.

Table 4.9: Estimated physical transport parameters for Br^- in the 10 macrocosm columns using the CDE transport model (± 95% confidence interval).

MIX	θ ($cm^3_{water}\ cm^{-3}_{pores}$)	λ (*cm*)	R^2	ρ ($g\ mL^{-1}$)
1	0.28 ± 0.00	3.14 ± 0.60	0.93	0.86
2	0.20 ± 0.01	2.46 ± 0.85	0.99	0.32
3	0.25 ± 0.01	3.53 ± 0.32	0.99	0.65
4	0.25 ± 0.02	2.67 ± 1.04	0.81	0.31
5	0.29 ± 0.02	2.21 ± 0.65	0.85	0.68
6	0.18 ± 0.01	3.69 ± 0.84	0.94	0.63
7	0.22 ± 0.00	5.33 ± 0.97	0.88	0.42
8	0.27 ± 0.02	2.25 ± 0.98	0.68	0.53
9	0.23 ± 0.12	6.42 ± 0.13	0.89	0.33
10	0.18 ± 0.01	4.26 ± 1.16	0.95	0.32

4.4.2.2 Metalaxyl, bentazone, isoproturon and linuron BTCs

Transport of metalaxyl, bentazone and isoproturon (both observed, as well as simulated using the CDE model or Monod model) through the experimental columns is shown in Figure 4.8, in terms of relative concentration versus the number of pore volumes (PV) eluted. In the case of metalaxyl, none of the BTCs could be described with the classical CDE model. As in the microcosms, delayed degradation appears to occur after a certain period of time. In the first part of the breakthrough curve the biomass is constant. However, after reaching a maximum effluent concentration, there is an exponential increase in biomass which leads to an increase in pesticide degradation and a decrease in effluent concentration. The Monod model[137] described the BTCs of metalaxyl well with R^2 varying from 0.81 to 0.97. The lowest goodness-of-fit was observed for metalaxyl in mix 9 (Table 4.10). Bentazone BTCs (Figure 4.8C, D) in mix 1 and 4 were well described using the CDE model with first-order kinetics (R^2 = 0.87 - 0.90), while mix 2, 3 and 5 showed more fluctuations in the effluent concentration, which decreased R^2 (0.59-0.60). This in contrast to the BTCs of mixes 6-10 which displayed delayed degradation and were well described with the Monod model (R^2 = 0.74-0.98). The classical CDE model could not be applied to the BTCs of isoproturon in contrast to the BTCs in the microcosm experiment (Figure 4.8E, F). The Monod model, however, described the isoproturon BTCs well for mix 1-6, as reflected in the values of the determination coefficient R^2 (0.77 – 0.96) (Table 4.10). On the other hand, the Monod model was not able to describe the BTCs of isoproturon in mix 7-10. The effluent concentration was very low in these mixes, with a removal of about 98% of the initial concentration. Linuron, which is the most immobile pesticide from the pesticides studied, did not breakthrough in the macrocosm system. No detectable concentrations could be measured in the effluent of any mix even after 150 days. Linuron is known as a substrate for bacterial growth[159,160] and as a pesticide

with a low mobility[156-158]. A combination of both factors could explain the absence of linuron in the effluent.

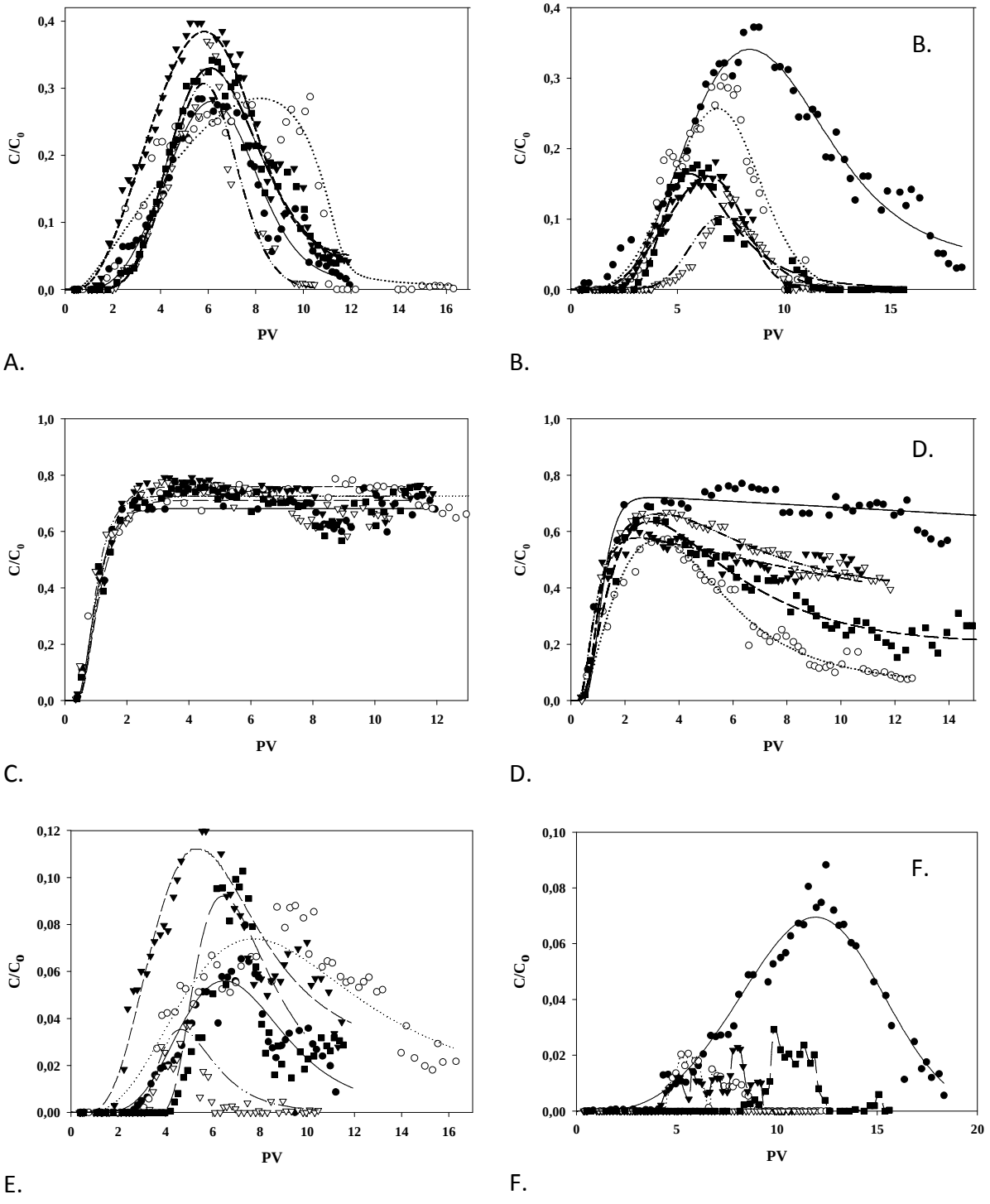

Figure 4.8: Observed and simulated BTCs of metalaxyl (A., B.), bentazone (C., D.) and isoproturon (E., F.) in 10 experimental column set-ups containing different types of organic substratums. Observed BTCs are presented with the following symbols in A: ● , mix 1; ○ , mix 2; ▼ , mix 3; ▽ , mix 4; ■ , mix 5. Simulated BTCs are presented as: ——— , mix 1; ····· , mix 2; — — , mix 3; —·— , mix 4; ——— , mix 5 and in B: ● , mix 6;

○, mix 7; ▼, mix 8; ▽, mix 9; ■, mix 10. Simulated BTCs are presented as: ——, mix 6; · · · · · · · , mix 7; — —, mix 8; —— · ·, mix 9; ——, mix 10. Relative concentrations are plotted against the number of pore volumes (PV).

4.4.2.3 Sorption parameters of metalaxyl, bentazone and isoproturon

The solute reaction parameters of metalaxyl, bentazone and isoproturon in the different mixes are presented in Table 4.10. The Freundlich parameter K_f for metalaxyl has slightly lower values in the macrocosm experiment compared to the microcosm experiment, which could be attributed to a lower density of the organic matrix. The Freundlich exponent *n* has mostly values > 1 which is similar to what has been observed in the microcosms. The *n* value in mix 2 is extremely high and has a high standard deviation which is probably the result of the poor fit to the observed data or caused by the high correlation between the K_f and *n* value.

In agreement with the results obtained in the microcosms, retention of bentazone in the organic mix is minimal ($K_f < 88.17 \cdot 10^{-3}$ *L* kg^{-1}). Its low sorption potential was very likely on the basis of its high solubility[168]. As in the microcosm study, the value of the Freundlich exponent of bentazone was proven to be highly uncertain.

The Freundlich parameters, K_f and *n*, which describe the sorption of isoproturon, have a higher standard deviation than the standard deviations associated with the Freundlich parameters derived from the microcosms study. Due to the low isoproturon concentration in the effluent, the accuracy of the analysis decreases, which leads to a larger variability and therefore to a less precise fit for *e.g.* mix 4 and 5. The sorption capacity of the different mixes appears to be quite similar and comparable to those obtained in the microcosm system, which makes extrapolation of the results to a larger scale possible. Finally, according to their retention strength, pesticides could be classified as follows: linuron > isoproturon > metalaxyl > bentazon.

Table 4.10: Predicted sorption parameters (K_f, n), first-order degradation constant (μ_l) and determination coefficient (R^2) of metalaxyl, bentazon and isoproturon (± 95% confidence interval)

Mix	Metalaxyl			Bentazon				Isoproturon		
	K_f [$L\ kg^{-1}$]	*n*	R^2	K_f (x 10^{-3}) [$L\ kg^{-1}$]	*n*	μ_l (x 10^{-3}) [h^{-1}]	R^2	K_f [$L\ kg^{-1}$]	*n*	R^2
1	1.24 ± 0.10	1.19 ± 0.08	0.96	4.30 ± 3,80	2.25 ± 4.08	1.09 ± 0.05	0.74	2.23 ± 0.08	1.09 ± 0.05	0.84
2	0.82 ± 0.26	1.87 ± 0.21	0.93	0.10 ± 0.47	4.41 ± 2.01	1.51 ± 0.04	0.98	1.10 ± 0.14	1.30 ± 0.13	0.82
3	0.68 ± 0.10	1.41 ± 0.09	0.94	23.87 ± 12.51	1.62 ± 2.45	0.94 ± 0.05	0.89	1.20 ± 0.13	1.19 ± 0.12	0.92
4	3.28 ± 0.18	1.12 ± 0.04	0.96	0.03 ± 0.00	4.77 ± 4.06	0.97 ± 0.03	0.97	4.41 ± 0.24	0.89 ± 0.36	0.80
5	1.72 ± 0.12	1.05 ± 0.05	0.95	3.10 ± 2.86	2.67 ± 2.57	1.14 ± 0.07	0.94	3.46± 2.79	0.80 ± 0.60	0.77
6	1.44 ± 0.17	1.11 ± 0.08	0.95	1.28 ± 0.39	0.19 ± 0.70	/	0.74	3.77 ± 0.54	1.27 ± 0.08	0.96
7	2.68 ± 0.14	1.22 ± 0.09	0.93	88.17 ± 43.59	1.80 ± 0.24	/	0.98	/	/	/
8	1.88 ± 0.11	1.30 ± 0.04	0.96	0.00 ± 0.03	5.88 ± 4.02	/	0.89	/	/	/
9	4.94 ± 5.20	1.04 ± 0.18	0.81	0.24 ± 0.41	4.30 ± 0.81	/	0.97	/	/	/
10	3.99 ± 0.21	0.85 ± 0.05	0.97	39.69 ± 43.17	1.95 ± 0.50	/	0.94	/	/	/

4.4.2.4 Degradation parameters of metalaxyl, bentazone and isoproturon

As the Monod kinetic parameters are not significant and highly correlated, degradation can only be discussed based on visual assumptions from Figure 4.8. In general, for metalaxyl an estimation of the dissipation efficiency can be made based on the intersection of the decreasing BTC with the X-axes, the mixes could be classified with decreasing degradation efficiency as follows: 9 ≥ 8 ≥ 7 ≥ 10 > 4 > 1 > 2 > 5 > 3 > 6. As delayed dissipation occurred in all mixes, it is hypothesized that metalaxyl degrading biomass was initially present in small numbers, however due to the continuous application of metalaxyl to the system an enrichment occurred which resulted into a drastic decrease in metalaxyl effluent concentration in the second part of the BTC[67,169,170]. BTC obtained with mix 1 and 3 which are identical to mix 1 and 4 in the microcosm system could be fitted with the Monod model while this was not the case in the microcosm. Mix 6 and 8, which have the same composition as mix 2 and 3 in the microcosm both showed delayed dissipation. The increase in residence time and the slower flow could give the microbial community more time to proliferate and could therefore enhance dissipation on a large scale. In the classical biomix developed in Sweden[16], the amount of soil used was 25%. Soil is added as the main source of pesticide degrading microorganisms. In this study, the most efficient mixes (*e.g.* 7, 9, and 10) only contained 5% of soil. Hence, reducing the amount of soil in the biopurification system does not appear to decrease the efficiency of the system. Simultaneously with the decrease in metalaxyl effluent concentration, another compound increased in concentration. This compound is probably, N-2,6-dimethylphenyl-N-methoxyacetyl-alanine, the main metabolite of metalaxyl in soil[171].

Dissipation of isoproturon, which could be described by the Monod model, was already high at the maximum value, but decreased further and in some cases reaches 0. This decrease in effluent concentration, could be caused by the development of an isoproturon degrading community or by sorption of isoproturon to the generated biomass. As the decrease in isoproturon more or less coincides with the decrease in metalaxyl concentration, it could be possible that due to the lower flow and the larger volume compared to the microcosms, more biomass developed which increased the possibility of sorption of isoproturon to the biomass.

Degradation of bentazone in mixes 1 to 5 could be described using first-order kinetics. The first order degradation constant μ_l is fairly low with an average $t_{1/2}$ of 26.25 *d* in mixes 1-5, which is slightly higher than the value ($t_{1/2}$ = 12 *d*) reported by Huber and Otto[168] in field soils. The high water solubility of bentazone is advantageous for microbial degradation. However, for microbial degradation to occur a sufficiently long residence time in the matrix solution is of major importance. As the water flow in the biopurification system is much higher than in the field, degradation will be limited while leaching is stimulated. Compared to the microcosm studies, the degradation constant was one magnitude higher on a macro scale. As degraded or retained bentazone cannot be distinguished, it could also be possible that due to the lower flow in the macro scale columns more bound residues are formed. Delayed dissipation of bentazone occurred in mixes 6 to 10, which was described with the Monod model. Again, no distinction could be made between degradation or sorption of bentazone by the biomass.

4.4.2.5 Mass balance and residual pesticide concentrations in the matrix

The mass balances of metalaxyl, bentazone, linuron and isoproturon in the system are presented in Figure 4.9. The mass balance was calculated using the total amount of pesticide brought onto the system, the total amount measured in the effluent, and the total amount MeOH extractable fraction in the organic matrix. The fraction of pesticides which could not be recovered was considered as degraded or non-extractable and will be referred to as dissipated fraction. Non-extractable pesticide residues are those residues which are not degraded and not-extractable by the used procedure due to various physical and/or chemical interactions between the compound and the soil structure including covalent bounding and strong ad(ab)sorption.

For **metalaxyl**, the majority of the input was present in the degraded or non-extractable fraction (75-93%). Mixes 7, 8, 9 are highly efficient, but mix 10 appeared to be the most efficient mix in retaining and degrading metalaxyl. This mix consists of all substratums and contains a high amount of lignin rich materials such as straw, willow chopping, coco chips to stimulate the growth of white rot fungi. On the other hand, it also contains garden waste compost, cow manure, soil, peat mix which are nutrients or habitats for bacteria able to degrade metalaxyl. 2-16% of the total amount of metalaxyl leached, with mix 9 having the lowest amount of metalaxyl in the leachate.

The majority of the applied amount of **bentazone** in mixes 1-6 could be found in the leachate (> 50%), while degradation or the formation of non-extractable residues were the main process of bentazone dissipation in mixes 7-10. This could be expected as the latter mixes demonstrated the highest dissipation in the BTCs. Mix 7 and 10 appeared to be the most efficient matrix in retaining bentazon with a significant lower leached fraction (> 68% non-extractable or degraded).

The majority (85-98%) of the applied **isoproturon** was degraded or present as non-extractable residue in the organic substances. This is in accordance with the results of El-Sebaï *et al.*[172] in a clay silt loam agricultural soil, where 45-67% isoproturon was mineralized and 30-51% bound residue. The mixes with the lowest dissipation are mixes 3, 4, and 5, which is in line with the results obtained for metalaxyl and bentazone. 2-13% (extractable with methanol) of the applied isoproturon was retained in the matrix. 0-5% isoproturon leached with the water, which is slightly lower than the amount leached of metalaxyl (a pesticide with similar mobility). For isoproturon, mix 9 appears to be the most efficient matrix in retaining and/or degrading isoproturon. Dissipation of isoproturon was also high in mix 10 with low effluent concentrations. Mixes 9 and 10 both contain cow manure, which could stimulate degradation of isoproturon. This is in accordance with the observations of Doyle *et al.*[154] who reported an enhanced dealkylation reaction of phenylureas (*e.g.* isoproturon) by manure. Microbial degradation is considered to be the primary mechanism for isoproturon dissipation from soil[173,173-175]. Bacterial strains such as *Sphingomonas* sp*., Arthrobacter* sp*.* that completely mineralize the isoproturon carbon ring into CO_2 and biomass, have been isolated from soils that have been exposed to isoproturon for long periods[176,177]. Also soil fungi are able to metabolize isoproturon to hydroxylated metabolites[178]. Castillo *et al.*[179] also observed a strong decrease of isoproturon in biobeds inoculated with the white rot fungus *Phanerochaete chrysosporium*. On the other hand, several authors found that large fractions of ^{14}C-labelled isoproturon were not extractable by

various organic solvents. Between 25-75% of the initially applied ^{14}C-isoproturon was converted to non-extractable residues following 2-3 months of incubation[174,180-184].

Linuron was mainly degraded or non-extractable (91.24-97.72%) in the organic mixtures studied. Differences in dissipation between the mixtures were very small. As mentioned previously, only 0.53-0.61% of the applied linuron could be detected in the effluent. Moreover, the amount of extractable linuron was also very low (2.17-8.19%). Microbial degradation of linuron was already reported by Cullington & Walker[159], Sorensen *et al.*[185], Walker & Thompson[43], Di *et al.*[186].

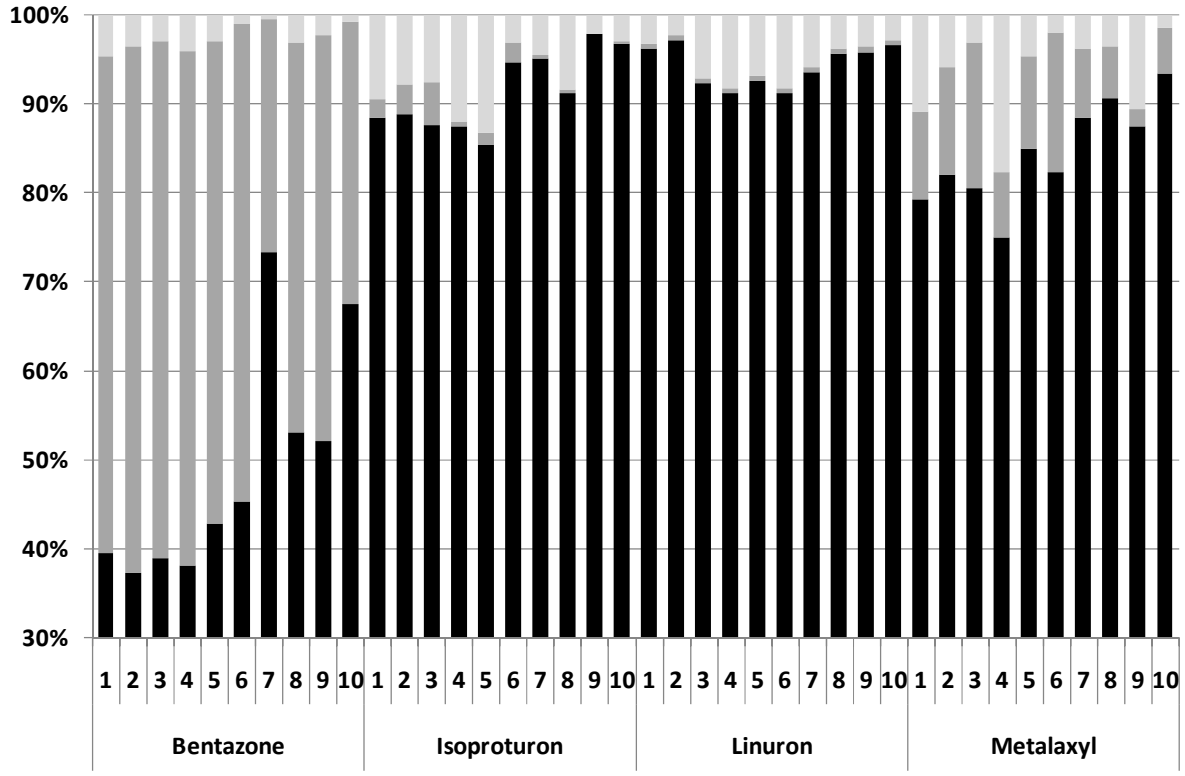

Figure 4.9: Mass balance of metalaxyl, bentazone and isoproturon in 10 experimental column set-ups containing different types of organic substratum. ■ = % dissipated or bound residues; ■ = % MeOH extractable fraction ■ = % leached.

Residuals amounts of metalaxyl, linuron, isoproturon and bentazone on three levels in the matrix are presented in Figure 4.10. Metalaxyl appeared to be retained in the top layer for mix 2, 4, and 9, while it is quite homogenously distributed for the other mixes (Figure 4.10A). Mix 2, 4, and 9 contain a higher amount of peat compared to the other mixes, which could lead to a strong sorption at the top where the pesticide enters the system (*cfr*. 3.3.4.2). Linuron, a rather immobile pesticide, is mainly retained in the upper layer of the column for all mixes, whereas the linuron concentration is very low at the bottom (Figure 4.10B). Isoproturon, a pesticide with similar mobility to metalaxyl, appears to be heterogeneously distributed in the column, probably also within one layer which could explain the high standard deviation (Figure 4.10C). Finally bentazone, a very mobile pesticide is quite homogeneously distributed in the column (Figure 4.10D).

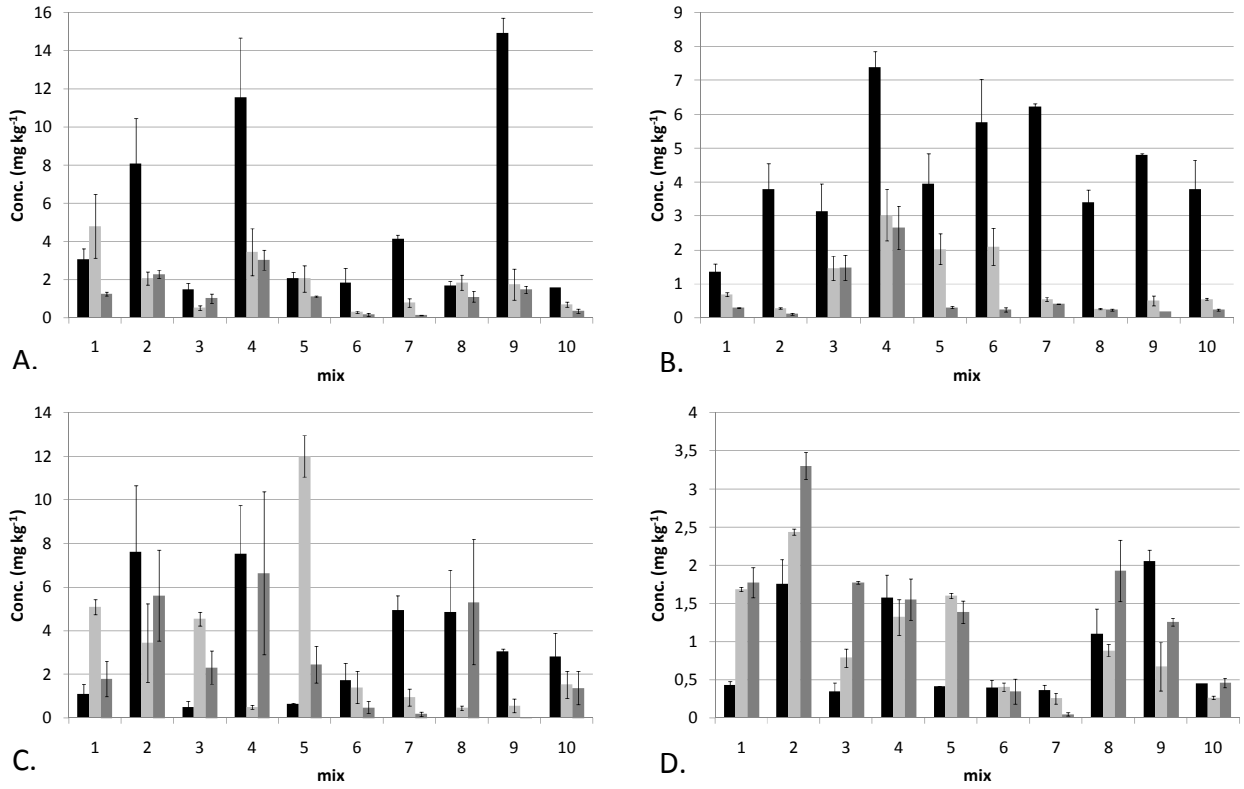

Figure 4.10: Residual pesticide concentrations (mg kg^{-1} dry matter (DM)) of A. metalaxyl, B. linuron, C. isoproturon, and D. bentazone in the organic substratum mixes on three levels of depth: ■ = upper part, ■ = middle part, ■ = lower part (error bars are ± standard deviation (P < 0.05).

4.4.2.6 Pearson correlation analyses

A Pearson correlation analysis ($p \leq 0.01$) was carried out between the percentage metalaxyl, bentazone and isoproturon leached in the macrocosms and the physicochemical characteristics (OM, EC, P_2O_5, K_2O, CaO, MgO presented in Chapter 2, Table 2.1 and the initial C/N ratio, %C, %N (Table 4.11)) of the different substratum mixes (Table 4.12). For metalaxyl, a significant negative correlation existed between the amount leached and the C content (R^2 = 0.637). This could be expected as an increasing amount of organic carbon, could increase the sorption of metalaxyl[187]. For bentazone, a high negative correlation existed between the amount leached and the N content (R^2 = 0.850). Mix 7 and 10, who appeared to retain bentazone the most, contained a higher amount of N (Table 4.11), which could stimulate bacterial degradation but could hamper the degradation by white rot fungi[188]. However, the main transformation of bentazone in soil appeared to be caused by bacterial activity[189], although little knowledge exists on specific bentazone degrading microorganisms. Finally, for isoproturon no significant correlations could be found.

Table 4.11: %C, %N and C/N ratio of the different mixes used in the macrocosms

Mix	% C	%N	C/N
1	5.29	0.17	32.06
2	16.98	0.40	42.44
3	5.59	0.17	33.88
4	23.26	0.42	56.05
5	7.52	0.20	38.54
6	5.68	0.32	18.02
7	19.22	0.92	21.01
8	9.41	0.35	27.26
9	28.45	0.71	40.07
10	26.23	1.02	25.84

Table 4.12: Pearson's correlation coefficients (r) between matrix characteristics and the percentage leached for isoproturon, bentazone and metalaxyl

	isoproturon	bentazone	metalaxyl
CN	0,096	0,540	-0,109
C	-0,492	-0,459	-0,637*
N	-0,523	-0,850*	-0,546
OM	-0,212	0,042	-0,467
EC	0,333	0,754	0,157
P2O5	0,383	0,458	0,461
K2O	-0,010	0,386	-0,278
CaO	0,374	0,354	0,418
MgO	0,371	0,348	0,496*

*correlation is significant at the 0.01 level (2-tailed)

4.4.2.7 Batch degradation experiments

Batch degradation experiments were carried out to obtain more information on the degradation potential of the organic mixes in the macrocosms after treatment. Four mixes were selected, *i.e.* mix 3 and 4 in which no apparent or the lowest degradation occurred for isoproturon, bentazone and metalaxyl, and mixes 9 and 10 which showed strong degradation for all pesticides studied. In addition, degradation experiments on fresh produced material with the same composition as mix 9 (9^*) were carried out. The $t_{1/2}$ and lag times recorded with various samples are presented in Table 4.13 for metalaxyl and linuron. Isoproturon and bentazone did not show any significant differences with the sterile control (results not shown), which indicates that no microbial degradation occurred. This is in contrast with the degradation occurring in the breakthrough curves. It could be possible that degradation of isoproturon and bentazon occurs through fungal degradation which could not be observed in the batch experiments. On the other hand, it could confirm the hypothesis that sorption to the biomass might occur.

For metalaxyl, it could be observed that degradation hardly occurred with samples from mix 3 and 9* with a half-life of 419 and 286 *d* in the upper and lower part of mix 3 and 190 *d* in mix 9* respectively. The half-life recorded for samples from mix 4, 9 and 10 was much lower than from 3 and 9^*, which indicates a higher degradation capacity in these mixes. As degradation is visible in the batch experiments, it indicates that a metalaxyl degrading

community might be present and that the decrease in effluent concentration can not completely be attributed to sorption of metalaxyl to the biomass. The half-life of metalaxyl in these mixes is comparable with those reported by Vischetti *et al.*[67] who found a decrease in $t_{1/2}$ with increasing application rate of metalaxyl on a biobed. In this study, metalaxyl was applied continuously which could enhance the development of a metalaxyl degrading community. Comparing $t_{1/2}$ of the samples of mix 4, 9, and 10 between the upper and the lower part, it could be noticed that degradation will occur faster in the upper part. Studies have shown that considerable in-field spatial heterogeneity in the distribution of the metabolizing community can exist[180]. The lower degradation capacity at the bottom could be caused by the presence of a smaller or different populations of metalaxyl degrading microorganisms due to the lower metalaxyl concentration at the bottom. In 4.4.3.5 it could be observed that a high metalaxyl residual concentration could be found in the upper part of mix 4 and 9 compared to the lower part where concentrations were low, although a higher degradation could be observed in the upper part in batch tests. The low residual pesticide concentration at the bottom could also be attributed to a combination of retention and degradation of metalaxyl in the upper part and thus not necessary indicate a high degradation rate at the bottom. The next parameter determining degradation is the lag time, this is the time period before the microorganisms start to degrade the compound. As degradation was a first-order process in mix 3, a lag time could not be determined. The lag time recorded for mix 4, 9, and 10 of samples from the upper part was smaller than this of mix 9*, indicating that a matrix previously treated with metalaxyl decreases the lag time. Differences in lag time for samples from the bottom and the upper part are small due to large standard deviations, thus it seems that adaptation of the microorganisms to the pesticide is not always spatially determined.

Linuron appeared to be degraded in all tested mixtures, which confirms the absence of linuron in the effluent. Half-lives of linuron in the upper and lower samples were similar for all mixes and did not significantly differ for 3 out of 4 mixes (Table 4.13). This is in contrast to the lag time recorded for bottom samples which is about 2.5-5 times higher than samples from the upper layer (see paragraph: Residual pesticide concentration in the matrix). As linuron is a fairly immobile pesticide, the majority will accumulate in the top layer. Due to the lower concentration present at the bottom, the microbial community will probably increase slower and therefore delays degradation in the batch tests. The observed half-lives of linuron are lower than the value ($t_{1/2}$ = 90 *d*) reported by Spliid *et al.*[80], probably due to the continuous supply of linuron in the current study which could have stimulated growth of the linuron degrading bacteria. The half-life and lag time in the fresh substratum mixture was higher than in the treated mix, which again points to the presence of a higher linuron degrading community in a matrix previously treated.

Table 4.13: $t_{1/2}$ and lag time of the degradation of metalaxyl and linuron recorded for samples from the upper (30-45cm) and lower (0-15cm) part of the macrocosm system 3, 4, 9, and 10 (± 95% confidence interval)

Metalaxyl				
Mix	**Upper part**		**Lower part**	
	$t_{1/2}$ (*d*)	**Lag time (*h*)**	**$t_{1/2}$ (*d*)**	**Lag time (*h*)**
3	419 ± 26	ND[b]	286 ± 19	ND
4	12 ± 2	60± 9	68 ± 29	128 ± 35
9	16 ± 6	85 ± 13	80 ± 30	41 ± 70
9[*, a]	190 ± 66	179 ± 17	/[c]	/
10	13 ± 1	62 ± 5	156 ± 36	25 ± 1
Linuron				
Mix	**Upper part**		**Lower part**	
	$t_{1/2}$ (*d*)	**Lag time (*h*)**	**$t_{1/2}$ (*d*)**	**Lag time (*h*)**
3	22 ± 5	85 ± 66	16 ± 6	218 ± 65
4	11 ± 4	67 ± 20	15 ± 5	206 ± 27
9	6 ± 0	79 ± 35	34 ± 10	260 ± 106
9[*]	45 ± 5	124 ± 117	/	/
10	21 ± 5	53 ± 49	26 ± 15	228 ± 16

[a] identical to mix 9 but made with fresh material
[b] not degraded
[c] not applicable

4.5 Conclusion

Pesticide displacement experiments were carried out for isoproturon, bentazone, metalaxyl, and linuron on small and large scale columns filled with different types of organic mixtures. The experimental bromide, metalaxyl, isoproturon, and bentazone BTCs were well described using the transport model based on the convection-dispersion equation with first-order degradation or with delayed dissipation using the Monod kinetics. The Monod kinetics described the breakthrough curves of the pesticides with delayed dissipation well, which might indicate that the decrease in effluent concentration is related to the growth in biomass. However, the model did not provide a meaningful value of the estimated degradation parameters as the initial biomass concentration was not known.

Freundlich adsorption parameters and degradation parameters were fitted to the observed BTCs. $K_{f,column}$ values fitted to transport experiments were much lower than those determined by previous batch studies, confirming the idea that sorption coefficients obtained from batch experiments are often not suitable for describing solute transport at the column or field scale. The K_{oc} value appeared to be a very good indicator of the mobility of the pesticide. Bentazone was the most mobile pesticide, followed by metalaxyl, isoproturon, and linuron.

Differences between micro- and macrocosms are the increased dispersivity and lower moisture content, which could be attributed to the increased column length and decreased flux respectively. The retention of the four pesticides was similar or in some cases slightly lower in the macrocosms compared to the microcosm system. The lower retention could be explained by the lower substratum density.

Delayed dissipation occurred for metalaxyl in the micro- and macrocoms, and also for isoproturon, and bentazon in the macrocosms. Hardly any breakthrough occurred for linuron, therefore this pesticide was classified as the most retained and/or degraded pesticide. Degradation of metalaxyl and linuron in the macrocosms was confirmed in batch experiments, while isoproturon and bentazon did not show any degradation. Hence, it was hypothesized that the increase in biomass enhanced sorption of bentazone and isoproturon which causes a decrease in the effluent concentration. Another possibility is that isoproturon and bentazon are degraded by white rot fungi, as suggested by others. In addition, degradation of metalaxyl and linuron appears to be more efficient in the upper part of the column, which was reflected in the lower half-live and shorter lag time for metalaxyl and linuron respectively. Furthermore, a continuous application of metalaxyl and linuron appeared to increase the efficiency of degradation considerably. Most mixes efficient in degrading or retaining pesticides were mixes containing dried cow manure. No significant differences could be found in retention and degradation of the mixes where no cow manure was added. Moreover, decreasing the amount of soil, compared to the amount used in a classical biomix, did not reduce the efficiency of the system. A removal in this mineral fraction would be advantageous as the cost of incinerating the material after use, increases with the soil fraction.

Chapter 5: Influence of pesticide-primed material on transport and degradation

5.1 Abstract

Laboratory column displacement experiments were performed to examine whether addition of pesticide-primed material to the matrix of an on farm biopurification system, intended to remove pesticides from agricultural waste water, positively affects the degradation of mobile pesticides in the system. Percolated column microcosms with varying types and amounts of metalaxyl and/or isoproturon primed material or non-primed material were irrigated with water artificially contaminated with isoproturon and/or metalaxyl. Transport of isoproturon was well described using the convection dispersion equation and no degradation was observed, even in columns inoculated with isoproturon-primed material. On the other hand, delayed dissipation of metalaxyl, i.e., after an initial lag phase, was encountered in all columns receiving metalaxyl. In all systems, degradation could be described using the Monod model, which might indicate that a metalaxyl degrading community grew in the systems. There was a clear correlation between the lag phase and the amount of metalaxyl-primed material added to the system, i.e., increasing amounts of added material resulted into shorter lag phases and hence more rapid initiation of growth-associated metalaxyl degradation in the system. Batch degradation tests further indicated that at the end of the experiment, isoproturon-degrading organisms and capacity were absent from all systems. In contrast, metalaxyl-degrading organisms were present in most systems but the highest capacity was found in the system inoculated with the highest amount of metalaxyl-primed material. Our observations suggest that indeed pesticide-primed material can reduce the start-up phase of degradation of mobile pesticides in a biopurification system and as such can increase its efficiency. However, the primed material should be chosen carefully and preferentially beforehand tested for its capacity to degrade the pesticide.

5.2 Introduction

Experiments described in Chapter 4, tested the influence of different organic substratums and various pesticides on the efficiency of the system on a small and large scale. These studies pointed out that a continuous application of the studied pesticides increased the degradation efficiency of the system (4.4.3.7). This phenomenon is explained by the proliferation of an adapted population able to degrade the applied pesticide. However, a major drawback is here that the performance of a biopurification system is often characterized by a relatively long lag phase or acclimation period during which significant amounts of the pesticide risk to escape treatment. This would be especially of relevance for highly mobile pesticides.

The lag time can be shortened by inoculating the system with sufficient amounts of active pesticide-degrading micro-organisms. Inoculation of the system might be performed with pesticide-primed soil or other pesticide-primed material. Pesticide-primed materials originate from agricultural field or other sources which have been long-term treated with and exposed to the target pesticide and which have developed a pesticide-degrading microbial community able to mineralize the compound. Such an inoculation approach has different advantages over inoculation with pure cultures[190-192]. Moreover, this approach would be a simple, low cost, practical and labour-extensive approach for inoculation. In addition to accelerate degradation during the start-up phase of the biopurification system, inoculation with pesticide primed soil would ensure complete mineralization of the target pesticides[192].

Sniegowski et al.[192] previously showed in a microcosm study that addition of pesticide-primed material indeed decreased the lag time of a biopurification system compared to inoculation with non-primed material and resulted into mineralization of the target pesticide. In that study, linuron was used as model target pollutant while linuron-primed soil was used as model pesticide-primed material. However, linuron is a pesticide with relatively low mobility displaying relatively high K_{oc} values of 410 to 620 $L\ kg^{-1}$. As a consequence, this pesticide will be retained relatively easily in a biopurification system due to sorption to organic material and as such will not appear in the leachate even when poor degradation occurs in the biopurification system. Therefore, the question remains whether inoculation of pesticide-primed material to the matrix of a biopurification system also affects the start-up phase of a biopurification system treating more mobile pesticides. This question was investigated in this study. We studied the fate of two fairly mobile pesticides (i.e. metalaxyl (K_{oc}= 47 $L\ kg^{-1}$) and isoproturon (K_{oc}= 36 $L\ kg^{-1}$)) in small scale biopurification systems inoculated with pesticide primed material and non-primed material. The pesticide primed material used in this study originated from the matrix of a biofilter, previously treated with metalaxyl and a field soil treated with isoproturon. The possible synergetic effect of the combination of the two inoculation sources and the mutual interactions between pesticides was considered.

5.3 Materials and methods

5.3.1 Selected pesticides, matrix description and column set-up

The pesticides used in this study were metalaxyl and isoproturon. Column microcosms were packed in triplicate with five different set-ups corresponding to five different mixes containing 45% (w/w) peat mix, 50% (w/w) straw, and 5% (w/w) of different inoculation materials. The different inoculation materials included (i) a reference sandy loam soil (non-primed soil) which was never treated with pesticides, (ii) material from a former biopurification system treating metalaxyl on a long-term base in Gembloux, Belgium and (iii) a field soil previously treated with isoproturon in Tielt-Winge, Belgium. This soil was treated every 3 years with isoproturon between 1989 and 2005. Mix 1 contained 5% of the reference soil, mix 2 contained 5% of the metalaxyl primed material, mix 3 and 4 contained 5% of the isoproturon primed soil and mix 5 contained 2.5% of both the metalaxyl and isoproturon primed material. The appropriate amounts of substratum were weighed, manually mixed in a bucket for about 5-10 minutes and then packed into the glass columns. Compaction of the matrix was carried out by placing a weight of 5 kg on top of the column. The columns were identical to the glass columns used in Chapter 4 (*l* x *d*: 15 *cm* x 10 *cm*).

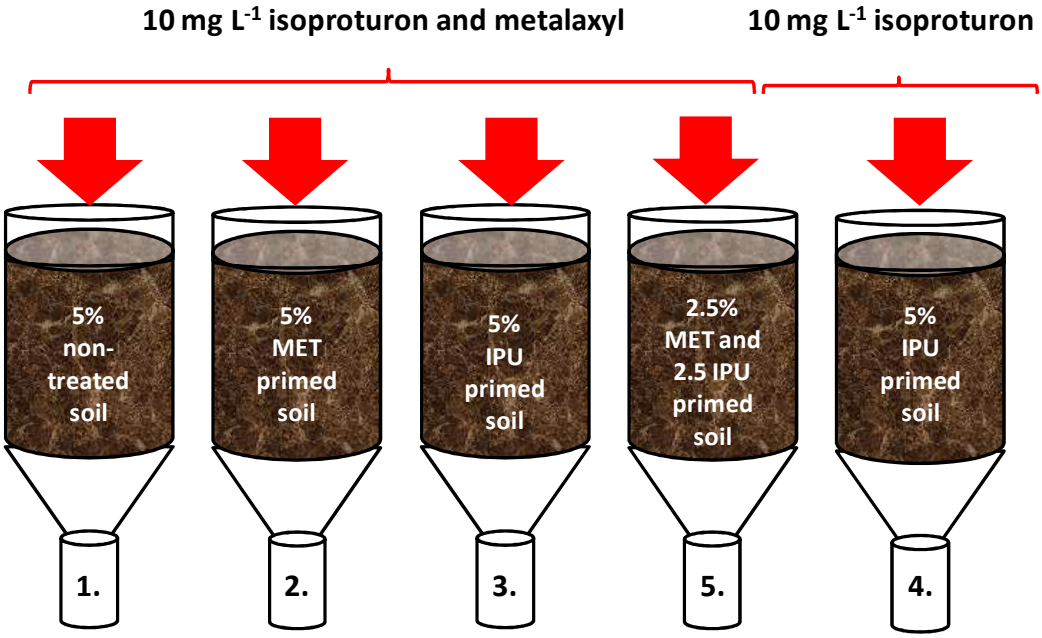

Figure 5.1: Experimental column set-up (MET: metalaxyl, IPU: isoproturon)

5.3.2 Displacement experiments

Displacement experiments were conducted under unsaturated, steady-state flow conditions similar to the small scale columns described in Chapter 4 (4.3.1.4). Steady state water flow conditions were established prior to the application of the solute step input. A $CaCl_2$ solution (0.001 *M* $CaCl_2$) was supplied to the column surface using PTFE (Polytetrafluoroethylene)

tubes. A peristaltic pump (Type 205S/CA, Watson Marlow, Zwijnaarde, Belgium) delivered a constant Darcy flux of 1.74 *cm* d^{-1}. It was assumed that steady-state conditions were reached once the mass of the column remained constant in time. When steady-state conditions were achieved, pesticides were applied to the column, initially together with a bromide solution (0.1 *mM* Br^-). The pesticide solution pumped onto the column from set-up 1, 2, 3 and 5 contained 0.001 *M* $CaCl_2$ and 10 *mg* L^{-1} isoproturon and metalaxyl, while the solution pumped onto columns of set-up 4 contained 0.001 *M* $CaCl_2$ and 10 *mg* L^{-1} isoproturon (Figure 5.1). The pesticide solution was added continuously as a step input, the bromide solution was applied as a pulse with a duration of 18.5 *h*. The effluent was collected in a fraction collector at the bottom every 2-3 days, outflow volumes and pesticide concentrations were measured (analysis of the studied pesticides with HPLC-DAD was described in 3.3.3). Bromide in the form of KBr was used as a non-reactive tracer to determine physical transport parameters. Bromide concentrations were determined by means of ion chromatography (Dionex ICS 2000), containing an AS15 column and KOH elluent. Bromide detection was performed by conductivity with a detection limit of 0.001 *mM*. The experiments lasted for about 224 days until the effluent concentrations of most pesticides reached a constant value.

5.3.3 Transport model

The transport model used is identical to the model used in Chapter 4 (4.3.2).

5.3.4 Batch degradation experiments

Batch degradation experiments were similar as in Chapter 4 (4.3.3). When the displacement experiment had ended, 0.500 ± 0.001 *g* of matrix was collected from the top layer of one replicate of each mix and transferred into an autoclaved Erlenmeyer. Experiments were carried out in triplicate for each mix. 50 *mL* of MMO medium (Mineral medium without any carbon source but with nitrogen containing salts) containing 20 *mg* L^{-1} of isoproturon and metalaxyl, which was prepared as described by Dejonghe *et al.*[138], was added to the Erlenmeyer of mix 1, 2, 3, and 5 and the same solution containing only 20 *mg* L^{-1} of isoproturon was added to mix 4. This mix was incubated on a shaker at 150 *rpm* at room temperature. A sterile control, to check for abiotic losses, was included for each mix through the addition of 8% chloroform to the MMO solution. Every 2-3 days 800 *µL* of the solution was sampled and filtered with a syringe filter containing a PVDF membrane with a pore size of 0.22 *µm* (Carl Roth, Karlsruhe-Rheinhafen, Germany). The aliquots were injected into the HPLC-DAD for pesticide concentration measurements.

5.4 Results and discussion

5.4.1 Breakthrough curves analyses

Physical transport was first evaluated by analyzing the bromide BTCs. The volumetric water content, θ, and the longitudinal dispersivity, λ ($D = \lambda v$), were fitted to the observed Br^- BTCs. To characterize retention and degradation of the pesticide in the mix, the Levenberg-Marquardt algorithm in HYDRUS-1D was used to analyze the BTCs of metalaxyl and isoproturon inversely. The physical transport parameters θ, q, ρ, and λ, were fixed during the inverse modeling of the pesticide BTCs. Estimated reaction parameters included the Freundlich parameters K_f and n, and the liquid degradation constant μ_l when first-order degradation was observed or the Monod kinetic parameters μ^*, μ_m^* (yield Y) and k_{decay} when delayed degradation occurred.

Average BTCs of Br^- through the five experimental column set-ups were fitted with the CDE model (R^2 = 0.90-0.96). The experimental BTCs did not show significant asymmetry or long-tailing that would indicate physical and/or chemical non-equilibrium. The overall mass recovery of Br^- was 95.2%, confirming physical equilibrium. The fitted transport parameters are presented in Table 5.1 together with the bulk densities of the columns. Equal water contents were found in mix 2, 4, and 5, while the water content of mix 1 and 3 was slightly lower compared to those of the other mixes. The moisture content is lower than those recorded in the previous microcosm experiment (4.4.2). These columns contained 25% soil, while in the present study the soil content is only 5%, resulting in a lower bulk density and a decreasing number of micropores and retained water. The low dispersivity indicates that the structure of the matrix in the column is quite homogenous[167].

Table 5.1: Estimated hydraulic parameters (θ, λ) determined with the CDE model, the determination coefficient R^2 and the bulk density ρ (± 95% confidence interval (n = 3))

MIX	Θ ($cm^3_{water}\, cm^{-3}_{pores}$)	λ (cm)	ρ (g mL^{-1})	R^2
1	0.29 ± 0.02	2.22 ± 0.41	0.27 ± 0.01	0.90
2	0.35 ± 0.01	1.19 ± 0.15	0.24 ± 0.01	0.91
3	0.27 ± 0.01	1.33 ± 0.31	0.28 ± 0.01	0.96
4	0.35 ± 0.02	1.53 ± 0.28	0.31 ± 0.01	0.94
5	0.35 ± 0.01	1.32 ± 0.16	0.27 ± 0.02	0.96

Transport of isoproturon and metalaxyl (both observed, as well as simulated using the CDE model or Monod model) through the experimental columns is shown in Figure 5.2, respectively, in terms of relative concentration (i.e. measured concentrations relative to the inlet concentration) versus the number of pore volumes (PV) eluted. All BTCs of isoproturon were very well described with the CDE model (R^2 > 0.96) and followed the classical pattern of

a BTC with a step input. This is in agreement with the breakthrough curves of isoproturon in previous microcosm experiments (4.4.2.1), but is in contrast with the results found on a macro scale where isoproturon showed delayed degradation (4.4.3.2). A reason for this contradiction could be the difference in flow rate which was applied in both systems. A lower flow in the macrocosm increases the residence time of isoproturon and will enhance the chance of isoproturon being degraded. Moreover, in the current experiment, only two pesticides were applied in contrast to Chapter 4, where a cocktail of 4 pesticides was used. The higher amount of pesticide input might contribute to a faster biomass growth. The higher biomass concentration might on his turn contribute to isoproturon sorption.

BTCs of metalaxyl (Figure 5.2B), on the other hand, were characterized by delayed dissipation. During the lag phase, the size of the original biomass present is not sufficient to initiate observable biodegradation. However, due to the continuous application of metalaxyl, proliferation of a metalaxyl-degrading biomass will occur in case metalaxyl is used as a C-source. When a critical content of metalaxyl-degrading biomass is obtained, this will results into observable degradation, which is clearly visible in the decrease in effluent concentration after reaching a maximum. This phenomenon has been observed in previous micro and macro column experiments in Chapter 4 and by Sniegoswki et al.[192]. The Monod model described the BTCs of metalaxyl well with R^2 varying from 0.83 to 0.96.

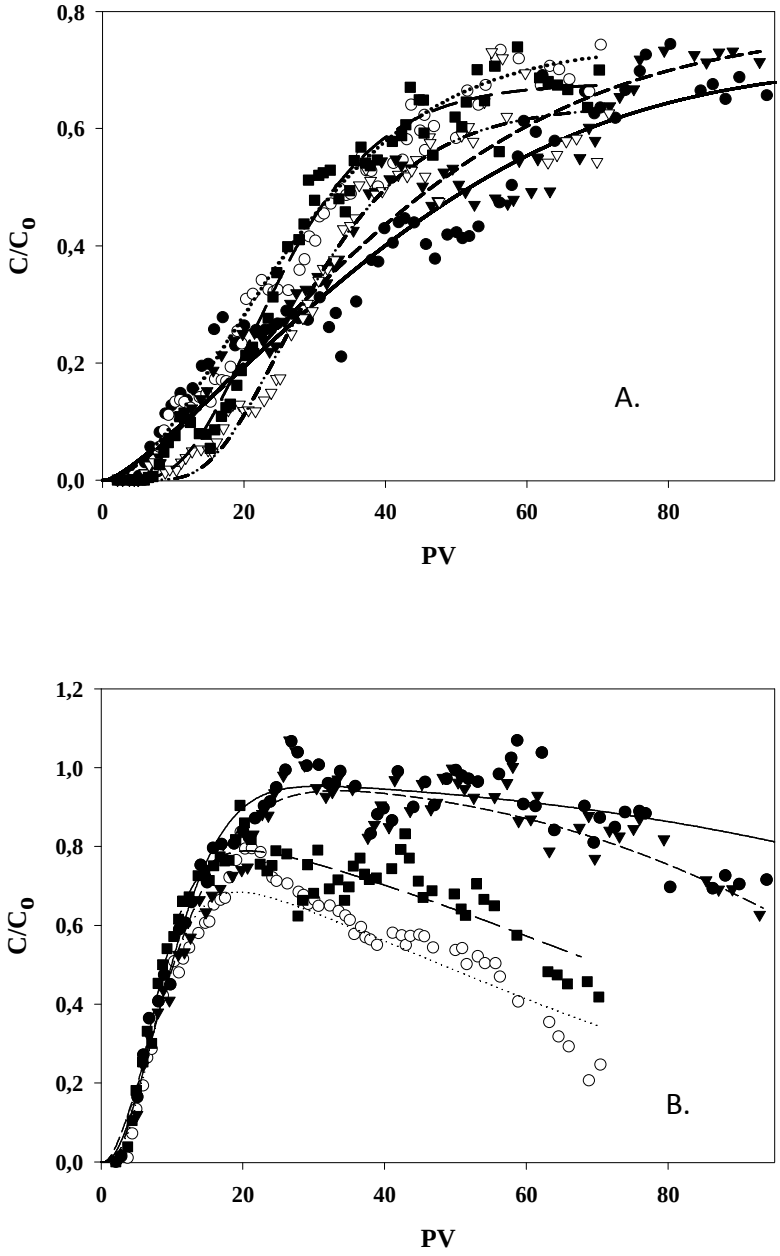

Figure 5.2: Observed and simulated BTCs of isoproturon (A.) and metalaxyl (B.) in 5 experimental column set-ups containing different types of inoculation sources. Observed BTCs are presented with the following symbols: ● , mix 1; ○ , mix 2; ▼ , mix 3; ▽ , mix 4; ■ , mix 5. Simulated BTCs are presented as: ——— , mix 1; ····· , mix 2; — — , mix 3; —·· , mix 4; ——— , mix 5. Relative concentrations are plotted against the number of pore volumes (PV).

5.4.2 Sorption parameters of metalaxyl and isoproturon

The solute reaction parameters of metalaxyl and isoproturon in the different mixes are presented in Table 5.2. The Freundlich parameter K_f of metalaxyl and isoproturon has slightly higher values than the ones obtained on a micro and macro scale in Chapter 4. This could be attributed to the lower amount of soil. As the peat fraction, which is rich in organic carbon (OC), is increased up to 45%, the OC content of the mix increases which could enhance sorption[187,187]. Differences in K_f values of isoproturon between mixes are quit large, but can be explained by a slight overestimation of the values in mix 4 and 5 and a slight underestimation of the retention of isoproturon in mix 1, 2, and 3. A better fit could however not be obtained. In accordance to the results in Chapter 4, *n* values of metalaxyl and isoproturon are higher than 1.

Table 5.2: Predicted sorption parameters (K_f, n), first-order degradation constant (μ_l) and determination coefficient (R^2) of metalaxyl and isoproturon (± 95% confidence interval)

	Metalaxyl			
Mix	K_f [$L\ kg^{-1}$]	***n***		R^2
1	3.00 ± 0.65	1.50 ± 0.09		0.91
2	6.41± 1.65	1.29 ± 0.12		0.83
3	2.55 ± 0.74	1.58 ± 0.12		0.96
5	5.97 ± 0.99	1.25 ± 0.08		0.93
	Isoproturon			
	K_f [$L\ kg^{-1}$]	**n**	μ_l (x 10^{-3}) [h^{-1}]	R^2
1	7.63 ± 1.04	1.78 ± 0.07	6.19 ± 0.48	0.96
2	14.02 ± 0.97	1.50 ± 0.06	4.04 ± 0.47	0.98
3	9.17 ± 1.11	1.66 ± 0.06	4.72 ± 0.47	0.97
4	36.25 ± 2.54	1.01 ± 0.04	6.15 ± 0.35	0.97
5	28.24 ± 1.96	1.12 ± 0.04	5.32 ± 0.26	0.98

5.4.3 Degradation parameters of isoproturon and metalaxyl

The degradation of isoproturon could be described with first-order kinetics. After reaching equilibrium, the amount of isoproturon was reduced with about 30% of the initial concentration (Figure 5.2B). The first-order constant was similar to this calculated in previous experiments (4.4.1). As we assumed that degradation only occurred in the liquid phase, the half-life can not be calculated from μ_l ($t_{1/2} = \ln 2/\mu_l$). $t_{1/2}$ should be calculated from $\ln 2/(\mu_l/R)$ (R= retention coefficient) for sorbing chemicals. In that case, the average $t_{1/2}$ value of isoproturon in the mixes was 298 days. In literature, isoproturon laboratory half-life ($t_{1/2}$) varies from 3 to 200 days according to soil properties[193] and water content[194].The higher observed half-life can probably be explained by the higher sorption of isoproturon to the organic matrix, which decreases the bioavailability. As the difference between the degradation constants was minimal between the various mixes, the addition of an isoproturon degrading soil was not a surplus. The highest degradation was observed in columns containing mix 4, which could be logical as this mix contained 5% isoproturon

primed soil and as no metalaxyl was applied which could reduce the number of isoproturon degrading microorganisms. However, this value was not significantly different from the control value where metalaxyl was added and a non-primed soil was used. Apparently, as the field from which the isoproturon primed-soil originated had not recently been treated with isoproturon, it is possible that the isoproturon degrading community decreased drastically and was therefore not able to function. The isoproturon-degrading capacity of that soil was verified by Sniegoswki et al. (personal communication) and indeed, only after a very long lag time of more than 60 days, isoproturon degrading activity was observed indicating that the isoproturon degrading capacity of that soil was initially low.

In the case of metalaxyl, it is visually clear that mix 2 is more efficient in metalaxyl degradation, followed by mix 5, mix 3, and mix 1 (Figure 5.2A). This is in accordance to what was hypothesized, mix 2 contained 5% metalaxyl primed material, mix 5 contained 2.5%, while mix 1 and 3 did not contain any metalaxyl degrading soil. A metalaxyl degrading microbial community was apparently already present in the reference and isoproturon treated soil although at very low amounts or the community present was able to adapt rapidly and acquire a metalaxyl-degrading capacity due to the continuous application of metalaxyl. Similar observations were reported for biopurification microcosms containing a non-primed soil when fed with linuron[192].

The fact that metalaxyl is more easily degraded than isoproturon in the presence of pesticide primed soil, may also be related to the type of pesticide. Sorensen *et al*.[180], observed that mineralization of recalcitrant pesticides such as the phenylureas, is restricted to a small group of organisms. This is in contrast to more easily degradable compounds such as MCPA that are metabolized by a broad range of soil microorganisms. Thus degradation of metalaxyl could be facilitated by a broad spectrum of microorganisms, in contrast to isoproturon, whose degradation apparently needs more specific types of microorganisms.

5.4.4 Batch degradation experiments

Batch degradation experiments were carried out with matrix samples taken at the end of the displacement experiment in order to obtain more information on the size of the degradation potential present at the end of the experiment in the different set-ups. Sniegowski et al.[192] previously showed that the lag phase in pesticide degradation kinetics could be related to the size of the pesticide-degrading community present in the tested sample. Samples containing relatively small sizes of pesticide-degrading biomass will exhibit relatively large lag phases, while samples containing relatively large sizes will exhibit a relatively small lag phase[192]. Isoproturon was added to all mixes, while metalaxyl was only added to mix 1, 2, 3 and 5. Degradation curves of isoproturon and metalaxyl are presented in Figure 5.3 for all mixes. The half-life and lag time recorded are presented in Degradation of isoproturon is presented in Figure 5.3B. Hardly any degradation occurred during the time period of about 46 days. A slight decrease was visible in the beginning which stabilized after about 17 days. This confirms the findings of the results of displacement experiment were hardly any degradation of isoproturon occurred. As mentioned above, it could be possible that the soil was treated too long ago, resulting into a large decay of the microbial community degrading isoproturon and as such, lost its capability of degrading isoproturon. Furthermore, it could

be concluded that also the reference soil did not develop the capacity to degrade isoproturon in the time frame studied.

Table 5.3. No degradation of metalaxyl occurred in the batches inoculated with mix 1, containing the reference soil, which is similar to the observations obtained in the column experiments were degradation of metalaxyl was minimal. Mix 2 appears to be the most efficient in degrading metalaxyl with a half-life of 4d and a lag time of 27h, indicating that indeed in this mix, the highest size of metalaxyl -degrading biomass was present. Differences in the lag phase were also observed between mix 3 and 5. Mix 5 which contains 2.5% metalaxyl degrading soil started degrading metalaxyl faster (39.63 *h* instead of 78.48 *h*) than mix 3 which contains only isoproturon primed soil. Since mix 3 also degraded metalaxyl, it indicates that this soil indeed contained a metalaxyl-degrading capacity. It can be concluded that the use of soil previously treated with metalaxyl, indeed increases the efficiency of degradation by inoculating with a larger size of pesticide-degrading biomass.

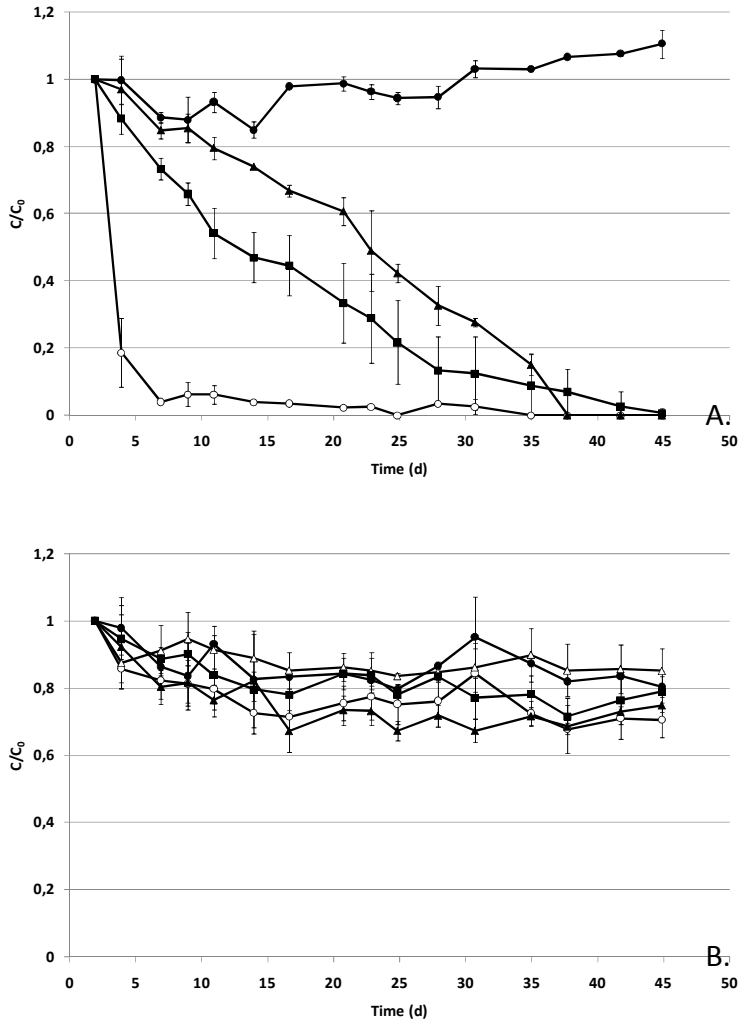

Figure 5.3: Degradation curves of metalaxyl (A.) and isoproturon (B.) in batch experiments with samples taken from the upper part of the columns of ● , mix 1; ○ , mix 2; ▲ , mix 3; △ , mix 4; ■ , mix 5 (error bars are ± standard deviation (p < 0.05), n = 3).

Degradation of isoproturon is presented in Figure 5.3B. Hardly any degradation occurred during the time period of about 46 days. A slight decrease was visible in the beginning which stabilized after about 17 days. This confirms the findings of the results of displacement experiment were hardly any degradation of isoproturon occurred. As mentioned above, it could be possible that the soil was treated too long ago, resulting into a large decay of the microbial community degrading isoproturon and as such, lost its capability of degrading isoproturon. Furthermore, it could be concluded that also the reference soil did not develop the capacity to degrade isoproturon in the time frame studied.

Table 5.3: $t_{1/2}$ and lag time of the degradation of metalaxyl (± 95% confidence interval)

Mix	$t_{1/2}$ (*d*)	Lag time (*h*)
1	ND*	ND
2	3.80 ± 0.01	26.76 ± 0.58
3	25.95 ± 0.24	78.48 ± 0.00
4	/*	/
5	22.41 ± 3.37	39.63 ± 5.05

*no degradation

*no metalaxyl applied to mixture 4

5.5 Conclusion

Pesticide displacement experiments were carried out for isoproturon and metalaxyl in small scale columns filled with a certain organic matrix composition containing peat mix, straw and a pesticide-primed material. This pesticide-primed material was organic matrix from a biopurification system treated previously with metalaxyl or field soil previously treated with isoproturon. The results show that matrix material from a biopurification system which has been in use for some years could serve as highly suitable material for the inoculation of newly starting systems in order to decrease the start-up phase. A biopurification system is most vulnerable to leaching during the first few months when the pesticide-degrading biomass has to be adapted and grow. From the performed experiments, it was observed that a removal of 40% in the metalaxyl concentration in the effluent was achieved after 131 days in the columns inoculated with pesticide-primed material, while the same removal in metalaxyl concentration was not yet achieved after 224 days in the columns containing non-primed material. Moreover, the total amount of metalaxyl leached decreased significantly from 68% in non-inoculated columns to 33% in columns inoculated with pesticide-primed material. Hence, the risk of leaching of some pesticides could be decreased by inoculating the matrix of a biopurification system with material from a well established biopurification system which had been used to treat similar pesticides.

Chapter 6: Transport and degradation of pesticides in a biopurification system under variable flux

6.1 Abstract

The efficiency of a biopurification system is to a large extent determined by the chemical and hydraulic load. A high input of pesticide contaminated water, might lead to a decreased retention and biodegradation of the pesticide. Therefore, insight into the behavior of pesticides under different fluxes in the biopurification system is necessary. The behavior of metalaxyl, bentazone, linuron, isoproturon and metamitron was studied under three different flows with or without the presence of pesticide-primed soil in micro- and macrocosm column experiments.

Non-equilibrium sorption prevailed at the highest flow for all pesticides (except linuron) in the microcosms. Degradation of the intermediate mobile pesticides in microcosms was also submissive to variations in flux. An increase in flux, leads to a decrease in retention, which in turn decreases the opportunity time for biodegradation. The presence of pesticide-primed soil was only beneficial for the degradation of metalaxyl.

Sorption and dissipation of the studied pesticides in the macrocosms was considerably higher compared to the microcosms. Breakthrough of isoproturon, linuron, metamitron and metalaxyl was small or not detectable. At increased flux, higher bentazone and metalaxyl concentrations were present in the effluent. According to the calculated mass balance, flux did influence dissipation of linuron considerably. Finally, it could be observed that incorporation of pesticide-primed soil had an influence, although minor, on pesticide degradation in the macrocosms.

6.2 Introduction

Experiments described in Chapter 4 and 5, tested the influence of different organic substratums and various pesticides on the efficiency of the system to reduce the pesticide effluent concentration on a small and large scale and the influence of the addition of a pesticide-primed soil on transport and degradation of isoproturon and metalaxyl on a small scale. All these experiments were performed at a fixed flux. To be able to optimize the efficiency of the biopurification systems on-farm, the influence of water flux should be assessed to minimize pesticide concentration in the effluent.

Insight into the expected hydraulic and chemical load is crucial for the control and management of the biopurification system. This load depends on the type of crop being treated, spraying scheme, behavior of the operator, the type of spraying machine, type of biopurification system and the period of the season (Chapter 1).

Firstly, the type of crop might determine the spraying frequency. Crops which are very submissive to pests and diseases (*e.g.* potatoes) should be frequently treated and thus will generate a higher hydraulic load. Secondly, depending on the spraying scheme, a farmer treating different crops with different pesticides will need to rinse often (and thus generate more contaminated water) to avoid contamination of the following crop with a remnant of the previous treatment. Thirdly, the attitude of the operator has to be taken into account. An operator not rinsing in the field will generate contaminated water with a much higher chemical load than an operator rinsing in the field. A fourth important parameter is the spraying machine. According to the type of sprayer (*e.g.* orchard sprayer or field sprayer) the internal (dead volume of the sprayer) and external chemical and hydraulic load will be different. The internal chemical and hydraulic load is higher in field sprayers as the booms and hoses are much longer compared to an orchard sprayer. However, the external contamination on the field sprayer is much smaller than on orchard sprayers due to the vertical spraying direction[195]. A fifth point of attention is the period of the season, which determines to a large extent how much contaminated water will be brought on the system. During winter, spraying of the crop is hardly performed, thus during late fall and winter no water will be generated. Finally, the type of biopurification system might also determine how much water can be processed. For example, a phytobac, can treat a larger amount of water compared to a biofilter as the dimensions of the phytobac are generally much larger and thus more substratum is present. An average hydraulic load on a biofilter is 20 $L\ d^{-1}\ m^{-3}$. However, a biofilter without the presence of a buffer tank (a tank where all the contaminated water is collected) will receive the load at once. This can mount up to 100 to 200 $L\ d^{-1}\ m^{-3}$, which is very pernicious for the efficiency of the system (Debaer, C., Personal communication)

To optimize the biopurification system, the application of different flow rates should be studied to determine its influence on the leaching of the pesticides. A high flow will probably decrease retention of the pesticide, which decreases the residence time and hence decreases the exposure time to biodegradation. On the other hand, a low flow does not permit to treat a high amount of pesticide contaminated water. The identification of a

suitable flow per m³ matrix will allow adjustments of the dimensions of the system according to the needs irrespective of the type or design of the biopurification system.

Therefore, the aim of this study was to test the influence of three different flows (low, intermediate and high flow) on transport and degradation of metamitron, bentazone, metalaxyl, isoproturon and linuron. Moreover, to verify the findings observed in Chapter 5, where the inoculation of a metalaxyl-primed soil was found to stimulate metalaxyl degradation, columns containing mixtures of five pesticide-primed soils in the matrix were compared to columns in which the matrix contained a non-primed soil which was not previously treated.

6.3 Materials and Methods

6.3.1 Selected pesticides, matrix description and column set-up

The pesticides used in this study were linuron, metalaxyl, bentazon, isoproturon and metamitron. Technical grade metamitron (99% purity) was kindly supplied by Agrichem B.V. (Oosterhout, The Netherlands). Methanol, acetonitrile, and water were of A.R. grade (VWR, Leuven, Belgium).

The substratums included in the columns are profoundly described in Chapter 2 and were peat mix, straw, dried cow manure, coco chips and on the one hand a mixture of pesticide-primed soil and on the other hand a reference soil (described in 2.3). The mixture of pesticide-primed soil consisted of a linuron-primed soil originating from a potato field in Halen, Belgium. The field was last treated in 2008. The isoproturon-primed soil was obtained from a wheat and oat field in Tielt-Winge, Belgium. This was last sprayed in 2005. The metamitron-primed soil originated from a sugarbeet field in Tielt-Winge, Belgium and was last sprayed in 2006. The bentazone-primed soil came from a maize field in Leefdaal, Belgium and was last sprayed in 2008. Finally, the metalaxyl-primed soil came from a potato field in Halen, Belgium. The final spraying date was not recorded.

Column micro- and macrocosms were packed in triplicate with the same mixture of air dried organic substratums for all treatments, i.e. 5% (w/w) dried cow manure, 25% (w/w) coco chips, 35% (w/w) peat mix, 25% (w/w) straw and 10% (w/w) soil. The used soil was the reference soil which had no pesticide treatment history or a mixture of a 2% metalaxyl, 2% isoproturon, 2% linuron, 2% metamitron and 2% bentazone primed soil. The matrix composition with pesticide-primed soil was used in the columns with low, intermediate and high flow. The reference soil was only incorporated in the columns and barrels which were irrigated at intermediate flow. Thus, for the intermediate flow six small and large scale columns were set-up (Figure 6.1). In this chapter, columns will be referred to as low, intermediate, high flow for the columns inoculated with pesticide-primed soil and as intermediate flow with the statement ‘with reference soil’ to indicate the difference with the pesticide-primed matrix.

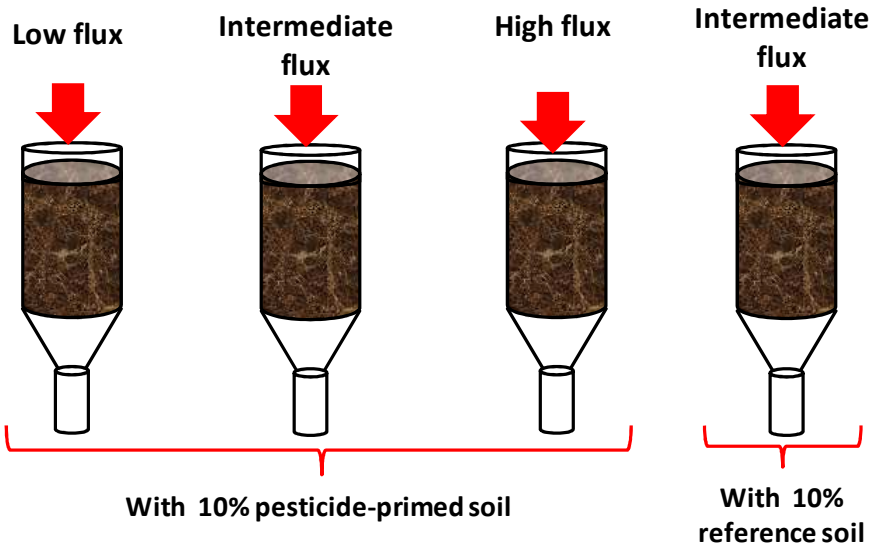

Figure 6.1: Experimental set-up of small and large scale columns

For the small scale columns, substratum amounts were weighed, manually mixed in a bucket for about 5-10 minutes to form homogeneous mixes, and then packed into the glass columns. Compaction of the matrix was carried out by placing a weight of 5 kg on top of the column. The columns were identical to the glass columns used in Chapter 4 and 5 (*l* x *d*: 15 *cm* x 10 *cm*). The large scale columns were as in Chapter 4 constructed in plastic barrels (polyethylene barrels, height: 50 *cm*, inner diameter: 45 *cm*). The air dried substratums were weighed and mixed with a concrete mixer to form homogeneous mixes, and then packed into the plastic barrels till a height of 45cm.

6.3.2 Displacement experiments

Displacement experiments were conducted under unsaturated, steady-state flow conditions similar to the columns described in Chapter 4 (4.3.1.4). Steady state water flow conditions were established prior to the application of the solute step input. A $CaCl_2$ solution (0.001*M* $CaCl_2$) was supplied to the column surface using PTFE (Polytetrafluoroethylene) tubes. A peristaltic pump (Type 205S/CA, Watson Marlow, Zwijnaarde, Belgium) delivered a constant Darcy flux of 0.84 *cm* d^{-1}, 1.45 *cm* d^{-1} and 2.40 *cm* d^{-1} for respectively the low, intermediate and high flow of the small scale columns or microcosms. The low, intermediate and high flux of the macrocosms was respectively 0.56 *cm* d^{-1}, 1.56 *cm* d^{-1} and 2.54 *cm* d^{-1}. Calculating the flow in function of the volume of the column leads to the following flows for the microcosms: 56.3 *L* d^{-1} m^{-3}, *95.6 L* d^{-1} m^{-3}, and *160.1 L* d^{-1} m^{-3}. For the macrocosms it results in the flows: 12.5 *L* d^{-1} m^{-3}, 34.6 *L* d^{-1} m^{-3}, and *56.4 L* d^{-1} m^{-3}. Hence the hydraulic and chemical load applied in the macrocosms is lower. For the sake of simplicity, the following notation will be used for the highest, intermediate and lowest flow, respectively, q_{max}, q_{mid} and q_{min}. To differentiate between intermediate flow in the columns with pesticide primed soil and with the reference soil, the notation of the columns with reference soil will be expanded to $q_{mid + ref}$.

It was assumed that steady-state conditions were reached once the mass of the column remained constant in time. When steady-state conditions were achieved, pesticides were, initially together with a bromide solution (1 *mM* Br^-), applied to the column. The pesticide solution pumped onto the columns contained 0.001 *M* $CaCl_2$ and 10 *mg* L^{-1} of each pesticide mentioned above. The pesticide solution was added continuously as a step input, the bromide solution was applied as a pulse with a duration of respectively 320 *h* and 380 *h* for the micro- and macrocosms. The effluent was collected in a fraction collector at the bottom every 2-3 days, outflow volumes and pesticide concentrations were measured (analysis of the studied pesticides with HPLC-DAD was described in 3.3.3). Bromide in the form of KBr was used as a non-reactive tracer to determine physical transport parameters. Bromide concentrations were determined by means of ion chromatography (Dionex ICS 2000), containing an AS15 column and KOH elluent. Bromide detection was performed by conductivity with a detection limit of 0.001 *mM*. The experiments lasted for about respectively 165 *d* and 180 *d* for the micro- and macrocosms until the effluent concentrations of most pesticides reached a constant value.

6.3.3 Transport model

The transport model used is identical to the model used in Chapter 4 (4.3.2) for BTCs where equilibrium sorption prevailed. However, non equilibrium sorption of certain pesticides could be observed. Transport of pesticides, where the sorption reaction is a rate-limited process, can be described with the one-site sorption model. As described in equation 4.1, transport of a pesticide for steady-state water flow conditions can be written as:

$$\frac{\partial C_l}{\partial t} = D\frac{\partial^2 C_l}{\partial z^2} - v\frac{\partial C_l}{\partial t} - \frac{\rho_b}{\theta}\frac{\partial C_s}{\partial t} - \mu_l C_l \qquad (6.1)$$

where *D* is the dispersion coefficient [$cm^2\ h^{-1}$], *v* is the pore water velocity [$cm\ h^{-1}$], $v = q/\theta$, in which *q* is the Darcian water flux [$cm\ h^{-1}$] and θ is the volumetric water content [$cm^3_{water}\, cm^{-3}_{pores}$], ρ_b is the bulk density [$g\ mL^{-1}$], μ_l is the first-order degradation constant for the solute in the liquid phase [h^{-1}], and *t* [*h*] and *z* [cm] are the temporal and spatial coordinates, respectively. The change in the sorbed concentration with non-equilibrium can be written as follows:

$$\frac{\rho}{\theta}\frac{\partial C_s}{\partial t} = \alpha\frac{\rho}{\theta}(C_{s,eq} - C_s) \qquad (6.2)$$

$$C_{s,eq} = K_d C_l$$

where α is a first-order kinetic constant describing the kinetics of the sorption process [h^{-1}] and $C_{s,eq}$ the sorbed concentration at equilibrium [$mg\ kg^{-1}$], C_s is the sorbed concentration of the kinetic sorption sites [$mg\ kg^{-1}$], and K_d the sorption distribution coefficient [$L\ kg^{-1}$].

Finally, incorporating (6.2) into (6.1) leads to:

$$\frac{\partial C_l}{\partial t} = D\frac{\partial^2 C_l}{\partial z^2} - \upsilon\frac{\partial C_l}{\partial t} - \frac{\rho_b}{\theta}\alpha(K_d C_l - C_s) - \mu_l C_l \qquad (6.3)$$

The model described above (referred to below as the one-site sorption model or the chemical non-equilibrium transport model) is described here as a first-order process depending only on pesticide concentration. However, as mentioned in Chapter 4 and 5, delayed dissipation of pesticides might occur. To describe this phenomenon, the simplified Monod kinetics will be incorporated as described in equation 4.7, 4.8 and 4.9 to describe BTCs where a lag phase was clearly present into HYDRUS-1D.

6.3.4 Batch degradation experiments

Batch degradation experiments were prepared as described in Chapter 4 and 5 (4.3.3). In this chapter, batch degradation experiments were carried out only for macrocosm samples. Samples (0.500 ± 0.001 *g*) of the matrix were collected from the top layer of one replicate of each mix and transferred into an autoclaved erlenmeyer. Experiments were carried out in triplicate for each mix. Samples were taken at different moments in time, more in particular, 12, 60 and 123 *d* after the start of the macrocosm experiment. This in order to evaluate lag time and half-life of pesticide degradation in time. 50 *mL* of MMO medium (Mineral medium without any carbon source but with nitrogen containing salts) containing 20 *mg* L^{-1} of isoproturon, metalaxyl, linuron, bentazone and metamitron, which was prepared as described by Dejonghe *et al.*[138], was added. This mix was incubated on a shaker at 150 *rpm* at room temperature. A sterile control, to check for abiotic losses, was included for each mix through the addition of 8% chloroform to the MMO solution. Every 2-3 days 800 *µL* of the solution was sampled and filtered with a syringe filter containing a PVDF membrane with a pore size of 0.22 *µm* (Carl Roth, Karlsruhe-Rheinhafen, Germany). The aliquots were injected into the HPLC-DAD for pesticide concentration measurements.

6.4 Results and Discussion

6.4.1 Influence of variable water flux on pesticide and tracer transport in microcosms

6.4.1.1 Bromide BTCs

As described in Chapter 4 and 5, physical transport was evaluated by analyzing the bromide BTCs. The volumetric water content, θ, and the longitudinal dispersivity, λ ($D = \lambda v$), were fitted to the observed Br^- BTCs. The experimental and fitted BTCs are presented in Figure 6.2.

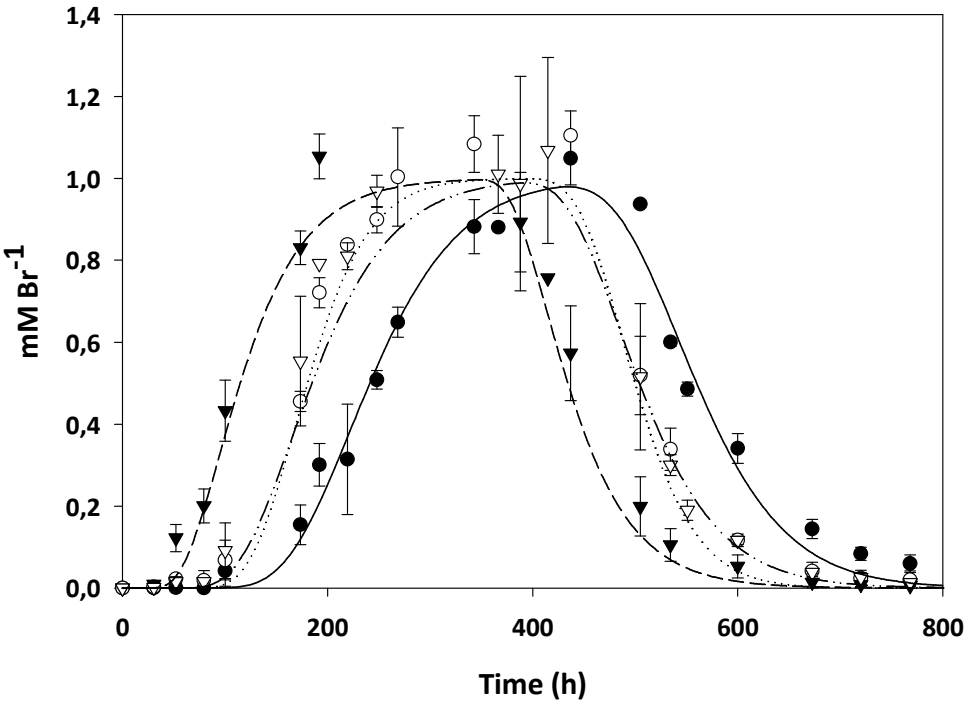

Figure 6.2: Observed and simulated BTCs of Br^- in 4 experimental microcosm set-ups with variable water flux. Observed BTCs are presented with the following symbols: ● , q_{min}, ○ , q_{mid}; ▼ , q_{max}; ▽ , $q_{mid + ref}$. Simulated BTCs are presented as: ——— , q_{min}; ····· , q_{mid}; — — , q_{max}; — · · , $q_{mid + ref}$. Absolute concentrations are plotted against time (h) (error bars are ± standard deviation($p < 0.05$) (n=3)).

The HYDRUS-1D model fitted the experimental data well with R^2 varying from 0.98 to 0.99. The overall mass recovery of Br^- was 104.97 ± 6.89% for the lowest flow, 99.15 ± 4.93% for the intermediate flow, 98.27 ± 11.31 % for the intermediate flow with reference soil and 96.49 ± 6.14% for the highest flow. The high recovery (> 100%) obtained at the lowest flow can be caused by analytical errors. Moreover, taking the standard deviation into account a value less than 100% can be obtained at the lowest boundary.

The influence of the different fluxes can be visually seen in Figure 6.2. The Br^- BTCs for q_{max} and q_{mid}, $q_{mid+ref}$ were markedly shifted to the left, compared to the Br^- BTCs obtained at q_{min}. Similar curves were obtained for q_{mid} and $q_{mid+ref}$. Peak shape and peak maximum was fairly similar for all flows.

Fitted transport parameters are presented in Table 6.1, together with the bulk densities of the columns. An increase of the flow rate, results in an increased water content of the organic matrix. This increased water content with increasing flux was also observed in chapter 4 and by Costa and Prunty[161] and Pot *et al.*[196]. At the start of the experiment, all barrels had a similar moisture content θ_z with a corresponding hydraulic conductivity $K(\theta_z)$. When the pesticide solution was added at three different flows higher than $K(\theta_z)$, than the water will enter the matrix faster than it flows out. Hence, the moisture content of the matrix will increase, which also increases the hydraulic conductivity $K(\theta)$. This process will continue as long as the moisture content of the matrix is smaller than the moisture content of the matrix when the hydraulic conductivity $K(\theta_q)$ equals flow q. At a certain moisture content θ_q is $K(\theta_q)=q$ and will the moisture content no longer be increasing. Therefore, a higher flow q is associated with a higher moisture content[197]. The composition of the organic matrix used in this chapter resembles most to the composition of mixture 5 in the microcosms in Chapter 4. The currently used mixture contains however a higher amount of peat (35% instead of 20%) and a lower amount of soil (10% instead of 25%). A comparison of the moisture content at q_{mid} with the moisture content in mixture 5 studied in chapter 4 at a similar flow (*i.e.* 1.74 $cm\ d^{-1}$) (Table 4.3), reveals that the moisture content in the current study was slightly higher in the columns with q_{mid} (0.77 $cm^3\ cm^{-3}$ compared to 0.64 $cm^3\ cm^{-3}$) studied in this chapter. This is probably caused by the higher fraction of peat mix as this has a high water holding capacity which reduces the flux.

Table 6.1: Estimated hydraulic parameters (θ, λ) determined with the CDE model, the determination coefficient R^2 and the bulk density (ρ) (± 95% confidence interval (n = 3))

Flow	θ ($cm^3_{water}\ cm^{-3}_{pores}$)	λ (*cm*)	ρ ($g\ ml^{-1}$)	R^2
q_{min}	0.59 ± 0.01	0.63 ± 0.12	0.36 ± 0.00	0.98
q_{mid}	0.75 ± 0.01	0.61 ± 0.16	0.37 ± 0.01	0.99
q_{max}	0.84 ± 0.02	1.21 ± 0.24	0.36 ± 0.004	0.99
$q_{mid+ref}$	0.79 ± 0.02	0.87 ± 0.36	0.32 ± 0.01	0.98

The dispersivity λ is within the range described by Jury *et al.*[198], who found that a typical value of λ is 0.5 to 2 *cm* for laboratory soil columns (Table 6.1). The dispersivity of the columns with q_{mid} is lower than the value obtained in mixture 5 in Chapter 4 (Table 4.3). A higher λ could indicate a higher heterogeneity of the pore system, which indicates that the columns in this chapter were packed more homogenously. A slight increase of the dispersivity λ could be observed with increasing flow rate, which is in accordance with the results of Vanderborght and Feyen[199], who showed that λ increases with increasing pore

water velocity in a sandy loam and loam soil and with those of Nützmann *et al.*[200], who described a linear relationship between pore water velocity and dispersivity in unsaturated porous media. This could indicate the presence of physical non-equilibrium (mobile-immobile (MIM) model), which can be described with the two-region, dual-porosity type solute transport[201]. The two-region concept assumes that the liquid phase can be partitioned into mobile (flowing), and immobile (stagnant) flow regions and that solute exchange between the two liquid regions can be modeled as a first-order process. A fit of the MIM model to the obtained experimental data of Br^- in the column with the highest flow, did however not improve the fit of the simulated curve to the observed data. The use of the MIM model to the Br^- BTCs at q_{max}, yields the following values for the estimated parameters: dispersivity λ was 1.15 (± 0.82) cm, the immobile water fraction (θ_{im}) was 0.05 (± 0.58) and the mass transfer rate coefficient (α) which describes solute exchange between the two liquid regions was 0.014 (± 0.35). The inaccurate determination of these parameters can be ascribed to the existence of an equilibrium condition between the mobile and immobile region. The BTC can at that moment also be described with the CDE transport model without immobile water. However the dispersion length is in that case a lumping parameter which also accounts for the exchange between the mobile and immobile region. Thus, in that case the dispersivity λ depends on the pore water velocity, and thus provokes a higher dispersion length for a higher water flux. The high recovery of Br^- also indicates that equilibrium between the two regions was reached.

The bulk density ρ is similar for all columns (Table 6.1). A slightly lower ρ was observed for the mixture containing the reference soil. As the soil is the only component which differs between the columns, the decrease in ρ will be caused by a lower ρ of the reference soil.

6.4.1.2 Metalaxyl, bentazone, isoproturon, linuron and metamitron BTCs

Transport of metamitron, metalaxyl, isoproturon and bentazone (both observed, as well as simulated) through the experimental columns is shown in Figure 6.3 and Figure 6.4. For all flows, linuron, the most immobile pesticide applied, could not be detected in the effluent or only at very low concentrations with a fairly high variation. This is in agreement with the observations in Chapter 4. Breakthrough of metalaxyl, bentazone, isoproturon and metamitron, could be described using the CDE transport model with equilibrium sorption or with the one-site sorption model, elaborated in Chapter 4 and in paragraph 6.3.3. Non-equilibrium sorption was taken into account as a higher sorption could be observed at a lower flow. This leftward shift with increasing velocity has been reported in several studies[141,202-206]. It is hypothesized that the higher total water content (the lower soil/water ratio) decreased the proximity of the solute molecule to the soil surface and so decreased sorption. The two-site sorption model, which assumes that the sorption sites can be divided into two fractions: sites where sorption is assumed to be instantaneous and sites where sorption is considered to be time-dependent, was not used[196]. This model did not provide a good fit and required the estimation of an additional parameter, f, which is the fraction of exchange sites assumed to be in equilibrium with the solution phase.

Dissipation of pesticides in the columns could be described using first-order degradation. Delayed dissipation occurred however for metalaxyl in the columns submissive to q_{max}. A higher flux and thus more metalaxyl input in the column, could lead to delayed dissipation, which was described with the Monod model (4.3.2).

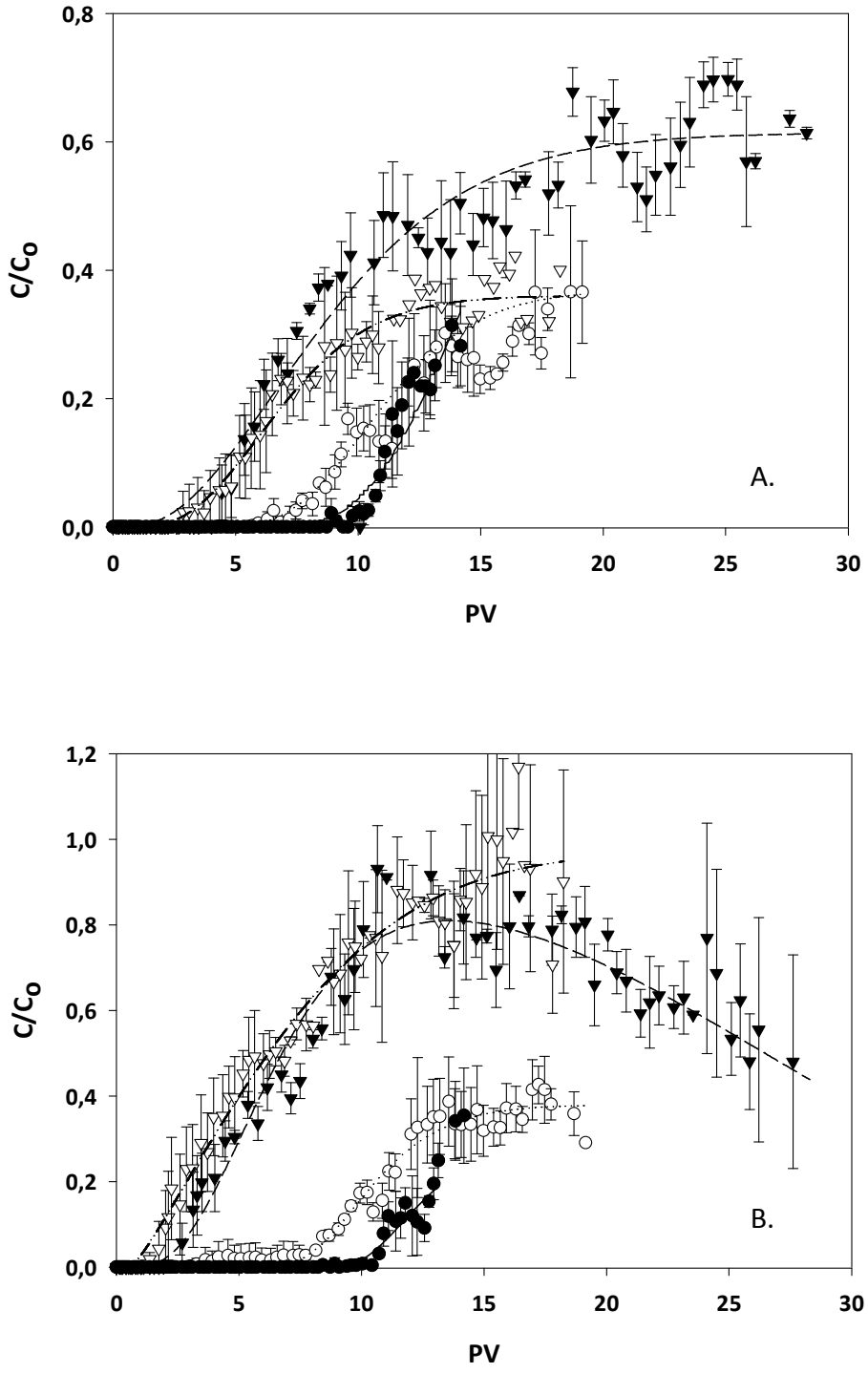

Figure 6.3: Observed and simulated BTCs of A. metamitron, B. metalaxyl in 4 experimental microcosm set-ups with variable water flux. Observed BTCs are presented with the following symbols: ● , q_{min}, ○ , q_{mid}; ▼ , q_{max}; ▽ , $q_{mid + ref}$. Simulated BTCs are presented as: ——— , q_{min}; · · · · · · · , q_{mid}; — — , q_{max}; ——· · , $q_{mid + ref}$. Relative concentrations are plotted against the number of pore volumes (PV) (error bars are ± standard deviation (P < 0.05) (n=3)).

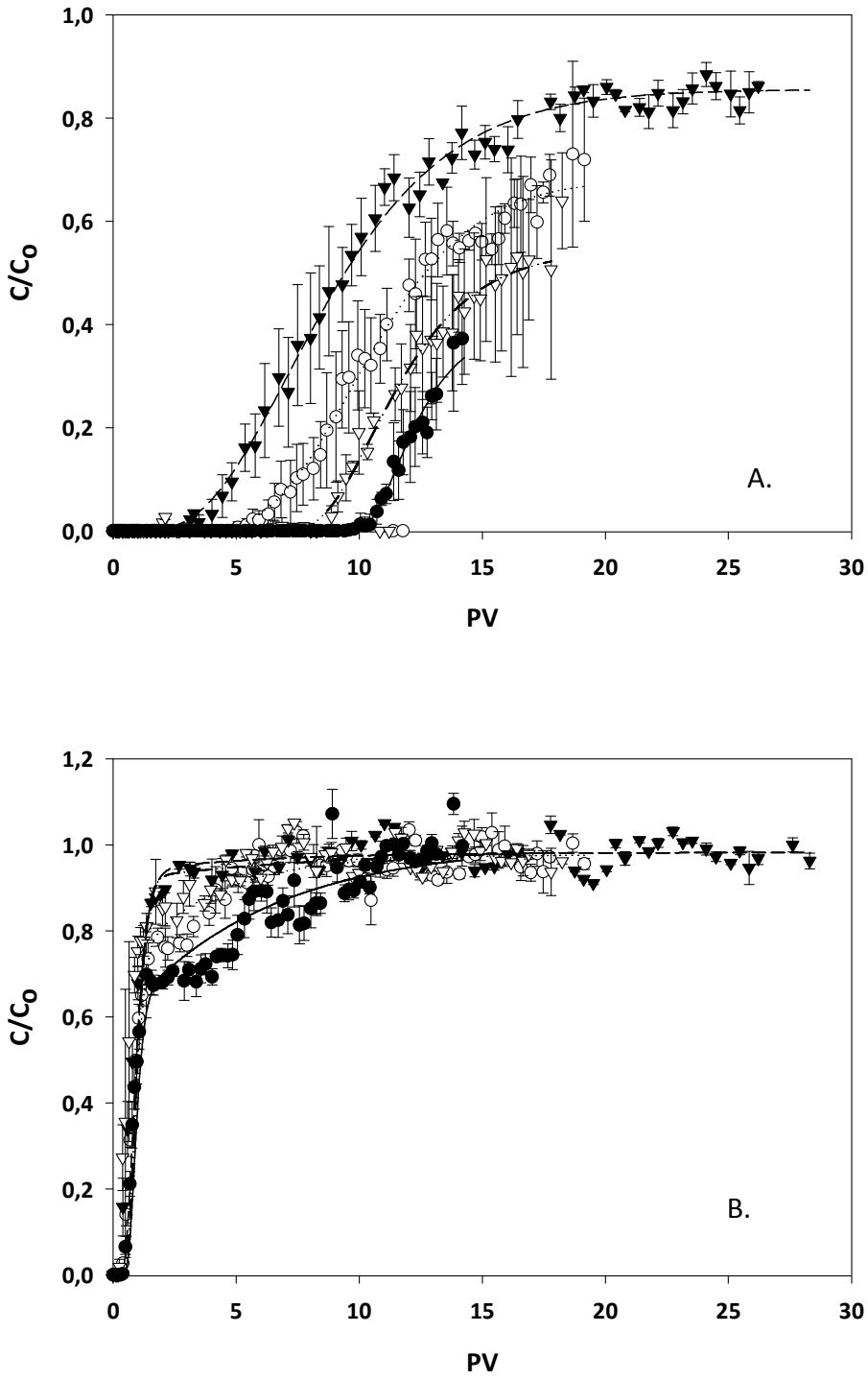

Figure 6.4: Observed and simulated BTCs of A. isoproturon, B. bentazone in 4 experimental microcosm set-ups with variable water flux. Observed BTCs are presented with the following symbols: ● , q_{min}, ○ , q_{mid}; ▼ , q_{max}; ▽ , $q_{mid + ref}$. Simulated BTCs are presented as: ——— , q_{min}; ····· , q_{mid}; — — , q_{max}; — · · , $q_{mid + ref}$. Relative concentrations are plotted against the number of pore volumes (PV) (error bars are ± standard deviation (P < 0.05) (n=3)).

Table 6.2 shows that the CDE model with equilibrium sorption and with one-site sorption was fitted to the experimental BTCs. A good fit to the observed data could be obtained for both models. R^2 ranged from 0.85 to 0.99 for the CDE model with equilibrium sorption and from 0.88 to 0.99 for the CDE model with one-site sorption. Hence, both models appeared to fit the experimental data well. However, for some pesticide-flow combinations, the fit was slightly higher with the CDE model with equilibrium sorption. The experiments performed at a faster flow, could be better simulated with the one-site sorption model, whereas the equilibrium model was adequate for the columns subjected to slower flows. This is in accordance with the results of Brusseau *et al.*[202]. The model which best fits the BTC is indicated in Table 6.2. The fact that transport of bentazone at all flows could be better described with the one-site sorption model, compared to the transport of other pesticides, could be caused by the fact that bentazone has a lower K_{ow} value. This is in accordance with Brusseau *et al.*[202], who stated that there was a positive relationship between the fraction of sorbent, for which sorption is essentially instantaneous, and the log K_{ow}.

Solute parameters (Table 6.2) were determined by inverse modeling of the BTCs. To fit the solute parameters, the physical transport parameters θ, λ, ρ, and q (Table 6.1) were fixed during the inverse modeling of the pesticide BTCs. The estimated sorption parameters for the CDE model with equilibrium sorption were the Freundlich coefficients K_f and n. When one-site sorption occurred, the estimated sorption parameters were the distribution coefficient K_d and the first-order kinetic constant α. To reduce the amount of estimated parameters, sorption was assumed to be linear (n was fixed to the value 1) in the case of non-equilibrium sorption. The estimation of n, which did not appear to vary extremely from 1, decreased the accuracy of the estimation of the other parameters.

The estimated degradation parameter was the first-order degradation constant μ_l. As previously mentioned, the Monod kinetic parameters μ^*, μ_m^*, and k_{decay} were not present due to their limited biological significance.

The BTCs of all pesticides (except for linuron and for metalaxyl at q_{max}) reached a plateau at q_{mid}, $q_{mid+ref}$, q_{max}. This indicates that a steady-state condition was reached and that the pesticide concentration in the effluent remained unchanged. This was however not the case for isoproturon, metamitron and metalaxyl at q_{min}, where the relative pesticide concentration was still increasing and did not reach a constant value after 165 *d.* A continuation of the experiment was however not an option as the columns were starting to get clogged, resulting in pounding water on top of the column.

6.4.1.3 Sorption parameters of metalaxyl, bentazone, isoproturon, linuron and metamitron

The estimated sorption parameters of metalaxyl, bentazone, isoproturon, and metamitron determined with the CDE with equilibrium sorption with the one-site sorption model are presented in Table 6.2. The water flux appears to have a significant influence on the sorption of metalaxyl, metamitron and isoproturon. As flow and thus pore water velocity increased, pesticides showed less retardation. This is in accordance with the results of Kim *et al.*[207] for the transport of benzene in a sandy soil and for the transport of simazine in unsaturated sand[208]. It is expected that the extent of sorption declines with an increment of the flow because the interaction time between the pesticide and the organic substratum decreases. This is also in accordance with the results of Pot *et al.*[196], where the K_d value of isoproturon decreased when rainfall intensities increased. At lower velocities, more sites seem to be at relative equilibrium with the solution, while at higher velocities, non-equilibrium sorption will prevail, causing a lower sorption of the pesticide to the organic substratum. Hence, pore water velocity does not influence sorption under equilibrium conditions[206], it does however influence kinetic sorption[209].

Non-equilibrium sorption mostly occurred in the columns at a flow q_{max}. However, non-equilibrium could also be observed for metalaxyl and metamitron at $q_{mid+ref}$, which is somehow out of the expectations as the only difference with the columns with flow q_{mid}, is the presence of 10% reference soil instead of a mixture of pesticide primed soil. The bulk density of the column with the reference soil was slightly lower (0.32 compared to 0.36), which is however not significant enough to account for the large difference in metalaxyl and metamitron retention.

Sorption of metalaxyl, metamitron and isoproturon to the organic matrix is quite similar. This was expected as those pesticides have a similar mobility. Sorption of metalaxyl and isoproturon to the matrix is higher than the sorption of the latter pesticides to mixture 5 in Chapter 4, where the applied flow is similar to q_{mid} and where a comparable composition of substratums is used. The higher sorption observed in this experiment could however be attributed to the higher fraction of peat (substratum with a high sorption capacity) used in the columns of this chapter.

Sorption of bentazone, a very mobile pesticide, is much lower compared to the other pesticides, which could be expected from previous results (Chapter 4). Due to the high mobility of this pesticide, the residence time of the pesticide in the column will be low, which decreases the time to achieve equilibrium sorption of bentazone with the organic matrix. A precise estimation of the sorption parameters of bentazone from BTCs also appeared to be difficult in Chapter 4, as breakthrough of this pesticide occurs very quickly and thus only a limited amount of data points is present to determine the sorption characteristics. Determining the Freundlich parameters with the CDE model with equilibrium sorption, leads to an inaccurate prediction which can be deduced from the large confidence interval (Table 6.2), a better prediction of the distribution coefficient could be obtained with the one-site sorption model, the estimation of the first-order kinetic constant α, was however also not very accurate as it showed high standard deviations.

Table 6.2: Predicted sorption parameters (K_f, n or K_d, α), and determination coefficient (R^2) of metalaxyl, metamitron, isoproturon and bentazone determined with the CDE model with equilibrium sorption or the one-site sorption model in columns submissive to different flow regimes with indication of the best fitted model (± 95% confidence interval) (n=3).

Pesticide	Flow	CDE with equilibrium sorption			CDE with one site sorption			Ideal model
		K_f [$L\ kg^{-1}$]	n	R^2	K_d [$L\ kg^{-1}$]	α [h^{-1}]	R^2	
Metalaxyl	q_{min}	27.90 ± 0.41	0.82 ± 0.03	0.97	25.76 ± 1.04	4.26 ± 54.14	0.93	Equilibrium
	q_{mid}	24.73 ± 1.14	0.91 ± 0.03	0.97	21.39 ± 0.87	3.45 ± 90.09	0.97	Equilibrium
	q_{max}	8.72 ± 2.53	1.24 ± 0.16	/	13.42 ± 0.49	$1.49\cdot10^{-2}$ ± $3.86\cdot10^{-3}$	/	One-site
	$q_{mid+ref}$	2.32 ± 0.40	1.85 ± 0.09	0.96	14.83 ± 0.86	$1.44\cdot10^{-4}$ ± $1.55\cdot10^{-4}$	0.97	One-site
Metamitron	q_{min}	25.43 ± 0.67	0.99 ± 0.02	0.95	24.49 ± 0.60	8.94 ± 10.51	0.95	Equilibrium
	q_{mid}	22.01 ± 0.58	1.02 ± 0.02	0.99	22,77 ± 0.30	0.15 ± 0.15	0.99	Equilibrium
	q_{max}	10.40 ± 1.61	1.36 ± 0.08	0.94	20.65 ± 0.89	$1.08\cdot10^{-2}$ ± $3.28\cdot10^{-3}$	0.95	One-site
	$q_{mid+ref}$	11.44 ± 0.99	1.24 ± 0.05	0.96	17.12 ± 0.48	$1.42\cdot10^{-2}$ ± $3.98\cdot10^{-3}$	0.97	One-site
Isoproturon	q_{min}	28.75 ± 0.39	0.92 ± 0.01	0.96	23.12 ± 1.34	4.22 ± 166.48	0.94	Equilibrium
	q_{mid}	17.62 ± 0.65	1.08 ± 0.02	0.99	20.14 ± 0.22	5.66 ± 73.90	0.99	Equilibrium
	q_{max}	13.68 ± 0.74	1.17 ± 0.03	0.97	19.69 ± 0.25	$2.27\cdot10^{-2}$ ± $4.11\cdot10^{-3}$	0.99	One-site
	$q_{mid+ref}$	26.05 ± 0.70	0.82 ± 0.02	0.99	20.35 ± 0.32	2.58 ± 58.28	0.98	Equilibrium
Bentazone	q_{min}	$2.76\cdot10^{-4}$ ± $3.87\cdot10^{-2}$	1.16 ± 62.15	0.85	3.32 ± 0.28	$8.77\cdot10^{-4}$ ± $0.13\cdot10^{-3}$	0.95	One-site
	q_{mid}	$2.05\cdot10^{-3}$ ± $1.97\cdot10^{-1}$	1.08 ± 45.17	0.91	1.55 ± 0.43	$1.82\cdot10^{-3}$ ± $0.61\cdot10^{-3}$	0.96	One-site
	q_{max}	$2.18\cdot10^{-5}$ ± $2.15\cdot10^{-3}$	0.98 ± 80.72	0.94	0.50 ± 0.80	$1.55\cdot10^{-3}$ ± $1.26\cdot10^{-3}$	0.95	One-site
	$q_{mid+ref}$	0.39 ± 0.23	2.21 ± 0.18	0.94	1.34 ± 3.07	$5.87\cdot10^{-4}$ ± $1.07\cdot10^{-2}$	0.88	One-site

6.4.1.4 Degradation parameters of metalaxyl, metamitron, isoproturon and bentazon

A first-order degradation rate μ_l was estimated although no degradation products were estimated. No experimental data about the potential decay processes in the column were present. However, degradation with the formation of metabolites of lower mobility than the parent compound, mineralization, or irreversible sorption of either the parent compound or metabolites could have occurred in the columns. The model used in this study assumed a total mass recovery of the chemicals so that all processes responsible for the loss of solute, which are not included in the governing equations, are lumped into the first-order degradation coefficient μ_l.

The half-life of the studied pesticides (except for linuron) is presented in Table 6.3. The half-life of the pesticide-flow combination is calculated using the first-order degradation constant of the CDE model with equilibrium or the one-site sorption model. The values presented in Table 6.3 are the values obtained with μ_l of the model which described the BTCs best.

As mentioned in paragraph 6.4.1.2, BTCs of metalaxyl, metamitron and isoproturon did not reach a steady-state condition yet at q_{min}, which does not permit an accurate estimation of the first-order degradation coefficient μ_l. The estimated half-life values of the BTCs of the latter pesticides at low flow rates were therefore omitted in Table 6.3.

Dissipation of **metalaxyl** appeared to decrease with increasing flow. However, due to the delayed dissipation, the relative metalaxyl concentration might reach the same values as transport of metalaxyl at q_{min} and q_{mid}. The same concentration level is at high flow however only reached after ± 27 PV, while at the lower flows, this level is reached after ± 15 PV. Thus during the start-up phase, which can last for 62 days in this case, metalaxyl leaching will be higher at a higher flow as the microbiota able to degrade metalaxyl did not fully develop yet. The higher flow reduces the residence time and decreases the opportunity for the community to decrease metalaxyl, however, due to the higher input of metalaxyl, the metalaxyl degrading community might be proliferated which finally reduces the metalaxyl concentration in the effluent drastically. From a visual comparison between degradation of metalaxyl at q_{mid} and $q_{mid+ref}$ in Figure 6.3B and from $t_{1/2}$ (resp. 55 and 578 *d*), it could be concluded that degradation was much lower in the column with the reference soil compared to the columns where metalaxyl-primed soil was included.

Table 6.3: Estimated half-lives $t_{1/2}$ (d) of metalaxyl, metamitron, isoproturon and bentazon in columns with variable flux (± 95% confidence interval) (n=3)

Pesticide	Flow	$t_{1/2}$ [d]	$t_{1/2}$ [d]
Metalaxyl	q_{min}	/	/
	q_{mid}	54.64	/
	q_{max}	Monod	Monod
	$q_{mid+ref}$	/	577
Metamitron	q_{min}	/	/
	q_{mid}	65.12	/
	q_{max}	/	74
	$q_{mid+ref}$	/	42
Isoproturon	q_{min}	/	/
	q_{mid}	166.72	/
	q_{max}	/	226
	$q_{mid+ref}$	91.94	/
Bentazone	q_{min}	/	3249696
	q_{mid}	/	403
	q_{max}	/	249
	$q_{mid+ref}$	/	1057

In accordance with metalaxyl, degradation of **metamitron** decreased with increasing flow (Table 6.3). Differences between half-lives are relatively small, as the retention of the pesticides is accounted for in the calculation of the half-life. However, in Figure 6.3A it is clear that degradation at a higher flow was lower, as the steady-state concentration of metamitron was significantly higher at the higher flow, while no significant differences could be observed between $q_{min,}$ q_{mid}, $q_{mid+ref}$. The lower degradation at higher flow may be caused by several processes operating simultaneously: (i) *q* might affect the density or distribution of the microbial biomass responsible for degradation, (ii) *q* might affect the nutrient concentrations in the organic matrix solution which may affect microbial degradation, (iii) *q* might affect the opportunity times that may be required for maximum degradation[210], (iv) the higher input of metamitron at the highest flow in the column might have toxic effects on the pesticide degrading community.

No significant differences could be observed between the steady-state concentration of metamitron at q_{mid} and $q_{mid+ref}$. Thus, although metamitron is sorbed to a lower extent to the organic matrix at $q_{mid+ref}$, the steady-state concentration at this flow is more or less equal to the steady-state concentration at q_{mid}. Hence, the matrix containing the reference soil could have gained the ability to degrade metamitron due to the continuous application of metamitron. It has been previously observed that this soil gained the ability to degrade metalaxyl and linuron (4.4.3.7). Microbial degradation of metamitron has previously been observed by Engelhardt and Wallnöfer[211], Charnay et al.[212], Parekh *et al*.[213], etc. Only very limited observations are present on fungal degradation of metamitron[214].

Comparing half-lives of metamitron obtained in the present study with values observed in other studies, show that the obtained half-lives are slightly higher than the values reported by Charnay *et al.*[212], who found an average half-live of 17 days in soil samples and Capri *et al.*[215] who found a half-live of 40 days at 10°C and 17.5 days at 30°C in soil. The higher half-lives found in this study could be caused by a negative effect of other pesticides on the metamitron degrading community or by a higher sorption of metamitron in the organic matrix compared to soil, which decreases the bioavailability. Finally, it could be noticed that simultaneously with the decrease in metamitron effluent concentration, another compound increased in concentration. This was also observed by Capri *et al.*[215] who identified it as 4-(dimethylimino)-3-methyl-6-phenyl-1,2,4-triazin-5(4H)-one.

The influence of water flux on **isoproturon** degradation is clearly visible in Figure 6.4A, where the steady-state concentration of isoproturon was highest for q_{max}, followed by q_{mid} and $q_{mid+ref}$. Slower degradation at higher fluxes can in this case probably also be explained by the process mentioned for the degradation of metamitron. Differences between q_{mid} and $q_{mid+ref}$ did not appear to be significantly different at higher pore volumes. From this we could conclude that the addition of isoproturon-primed soil did not provide a surplus value to the dissipation of isoproturon. Just as for metamitron, it could be possible that the reference soil gained the possibility to degrade isoproturon. A comparison of the half-lives of isoproturon (Table 6.3) with the results obtained in Chapter 4 (Table 4.4), shows that equivalent values were obtained in both experiments. However, higher first-order degradation constants and half-lives were found by Bending *et al.*[216] (μ_l = 0.66-0.016 d^{-1}) and Issa and Wood[194] ($t_{1/2}$ = 5-17 *d*) in soils.

Bentazone, which is hardly retained in the column is as a consequence of that, barely degraded. No significant differences could be observed in degradation of bentazone in the columns with variable flow in Figure 6.4B. This is probably the result of the small differences in retention between the fluxes. Differences in half-life are quite large, but have however no real significance, as confidence intervals on retention and first-order degradation coefficients are for some pesticide-flow combinations fairly high (Table 6.3). High values of $t_{1/2}$ were also found for the leaching of bentazone in the columns described in Chapter 4 (Table 4.5). In general, degradation of bentazone is negligible in the columns studied.

Linuron was not incorporated in Table 6.3 as no or little measurable concentrations could be detected in the effluent. This confirms previous results in Chapter 4, where breakthrough of linuron neither could be observed. As mentioned previously, a combination of a high retention and degradation potential of linuron could be the cause of linuron dissipation.

6.4.1.5 Mass balance of metalaxyl, metamitron, isoproturon, bentazone and linuron in the microcosm

The mass balance of metalaxyl, metamitron, isoproturon, bentazone and linuron is presented in Figure 6.5.

As could be expected, the majority of the applied **bentazone** was leached through the column and was recollected in the effluent (81.52-91.25%). Differences between the different fluxes are minor, which could already be observed in the shape of the BTCs. The amount of bentazone degraded or present as non-extractable residues in the organic matrix, is as a consequence very small and ranged from 8.52 to 18.20%. The lowest bentazone dissipation (8.52%), could be observed for the intermediate flow in the column containing the reference soil. This soil did normally not contain any bentazone-primed soil, which could cause a lower dissipation of this pesticide. Differences are however very small and the lower dissipation in the column $q_{mid+ref}$ could be coincidental as bentazone was shown to be a very persistent pesticide with little degradation in the biopurification system. Finally, it could be stated that the MeOH extractable fraction was extremely low (0.14-0.35%). The obtained results are in accordance with the results obtained in the macrocosm experiments described in Chapter 4.

From the mass balance of **isoproturon**, it could be concluded that an increase in flux results in a decrease in efficiency. The isoproturon concentration in the effluent is much higher in the column with flow q_{max} (49.27%) than in the column with flow q_{min} (6.54%). Moreover, the amount of MeOH extractable isoproturon varied from 1.64 to 2.78%, with a slightly higher retention in the column with flow q_{min}. The amount of dissipated isoproturon varied from 49.09-90.68%, with the highest degradation or formation of non-extractable residues at a flow of q_{min} due to the longer residence time of the pesticide.

As formerly described, **linuron**, the most immobile pesticide studied, showed negligible leaching through the column (0.19-0.91%). The fraction of retained linuron was also insignificant with the MeOH extractable fraction ranging from 0.13 to 0.61%. Thus, 98.96 to 99.46% of the applied linuron was incorporated in the organic matrix as non-extractable residue or was degraded. As the amount of linuron dissipated did not strongly differ between the different fluxes in the columns and as the presence of pesticide primed soil did not improve the efficiency of the system compared to the use of a non-treated soil (reference soil), it could be stated that for an immobile and reasonably easy degradable pesticide, such as linuron, the influence of water flux (within the range of fluxes studied) was of less importance.

The lowest effluent concentration and thus the highest efficiency of the small scale biopurification system for the treatment of **metalaxyl** could be obtained with a low flow (q_{min}). Dissipation of metalaxyl was 91.61% of the initially applied concentration, which is in the range of the results obtained in the macrocosms in Chapter 4 (4.4.2.5). Again, no differences could be made between the formation of non-extractable residues or degradation processes, however, as shown in the batch degradation experiments presented in Chapter 4 (4.4.3.7), degradation of metalaxyl can occur fast. In this case, considerable differences could also be observed between the use of a metalaxyl-primed soil and the

reference soil at the same flow. The use of the pesticide-primed soil increased dissipation of metalaxyl with 64.08%. This confirms the results obtained in Chapter 5.

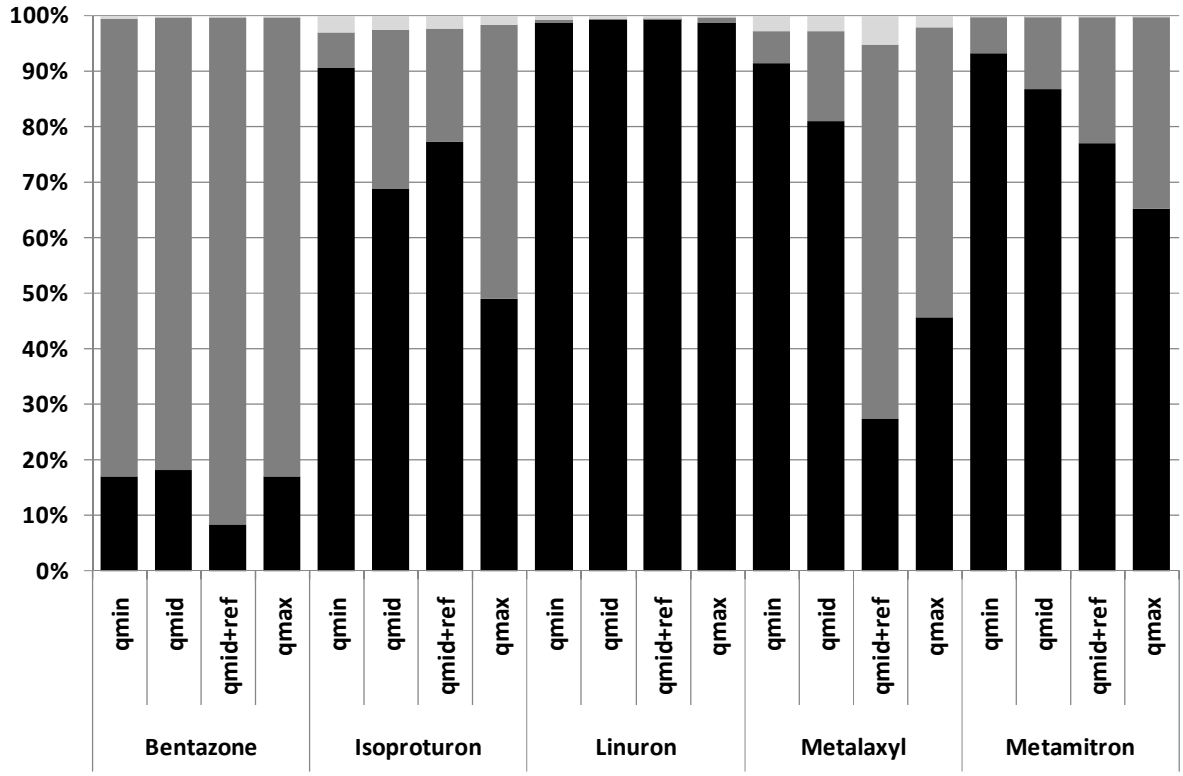

Figure 6.5: Mass balance of bentazone, isoproturon, linuron, metalaxyl, and metamitron in columns with variable flux. ■ = % dissipated residues; ■ = % MeOH extractable fraction ■ = % leached.

Finally, dissipation of **metamitron** was also most efficient at the lowest water flux, with a dissipation of 93.43% compared to 77.12% at the highest flux. The use of a pesticide primed soil increased dissipation slightly (9.75%) in comparison with the use of the reference soil at the intermediate flow. The MeOH extractable amount of metamitron was similar for all flows, but was extremely low (0.01%). This is in contrast to Charnay *et al.*[212] where 68.7 was dissipated (20.4% mineralized and 48.3% bound residues) and 28.3% of the initially applied metamitron was extractable. The experiment of Charnay *et al.*[212] only lasted 28 days, while metamitron in the microcosms was studied during 180 days. In this time frame, metamitron degradation and the formation of non-extractable residues could have increased.

6.4.2 Influence of variable water flux on pesticide and tracer transport in macrocosms

6.4.2.1 Bromide BTCs

The experimental and fitted BTCs of the inert tracer Br^- during transport in macrocosms at different fluxes are presented in Figure 6.6. The CDE model with physical equilibrium fitted the observed data well, with R^2 ranging from 0.91 to 0.98 (Table 6.5)

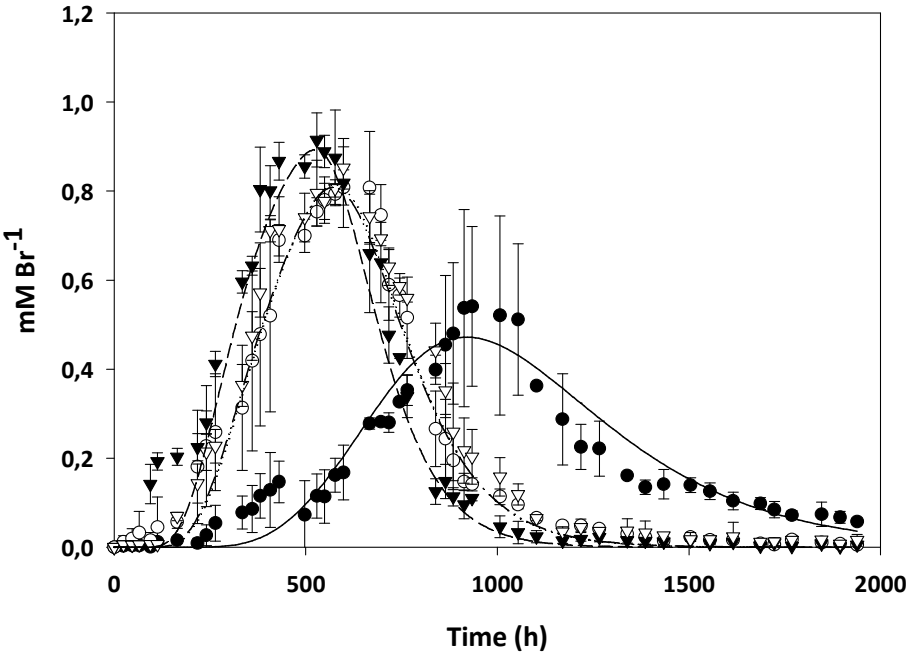

Figure 6.6: Observed and simulated BTCs of Br^- in 4 experimental macrocosm set-ups with variable water flux. Observed BTCs are presented with the following symbols: ● , q_{min}, ○ , q_{mid}; ▼ , q_{max}; ▽ , $q_{mid + ref}$. Simulated BTCs are presented as: ——— , q_{min}; · · · · · · · , q_{mid}; — — , q_{max}; ——· · , $q_{mid + ref}$. Absolute concentrations are plotted against time (h) (error bars are ± standard deviation (P < 0.05) (n=3)).

The fitted transport parameters and bulk densities are presented in Table 6.5. An increase of the flow rate, resulted in an increased water content of the organic matrix, which is in line with the observations of the microcosms. The composition of the organic matrix used in this study is not really similar to a mixture used in the macrocosm study in Chapter 4, where a flow of 0.90 *cm* d^{-1} is applied. Mixture 10 used in Chapter 4, however, comes closest to the composition of the current mixture. The current mixture contains however no compost and willow chopping and contains more peat mix (10% more), more coco chips (5% more) and more soil (5% more). The water content θ is higher in the current study at the lowest flow than the values obtained for the macrocosms in Chapter 4. The mixture used in this chapter contains more water adsorbing substratums such as peat mix and coco chips, which could increase the water content.

Table 6.4: Estimated hydraulic parameters (θ, λ) determined with the CDE model, the determination coefficient R^2 and the bulk density (ρ) (± 95% confidence interval (n = 3))

Flow	θ ($cm^3_{water}\,cm^{-3}_{pores}$)	λ (cm)	ρ (g mL^{-1})	R^2
q_{min}	0.47 ± 0.01	3.86 ± 0.37	0.43 ± 0.03	0.91
q_{mid}	0.61 ± 0.01	3.64 ± 0.32	0.39 ± 0.01	0.98
q_{max}	0.80 ± 0.01	3.54 ± 0.47	0.37 ± 0.04	0.98
$q_{mid+ref}$	0.60 ± 0.01	3.85 ± 0.40	0.38 ± 0.01	0.98

As expected, the value of the dispersivity λ is in the same range as the values obtained in Chapter 4. Differences in dispersivity between the different flows is insignificant, which is in contrast to the observations on a small scale. An invariant λ pointed out that physical equilibrium prevailed.

The variation in the bulk density ρ was slightly higher in the macrocosms. Due to the larger volumes, heterogeneity in the packing could increase.

6.4.2.2 Metalaxyl, bentazone, isoproturon, linuron and metamitron BTCs

Observed and fitted transport of bentazone and metalaxyl in macrocosms subjected to three different input flows are shown in Figure 6.7 and Figure 6.8, respectively. Breakthrough of isoproturon, linuron and metamitron was low or did not occur. Breakthrough of isoproturon at q_{max} occurred after about 7.9 PV, and reached a maximum relative concentration (C/C_o) of 0.09. After about 11.6 PV, the relative concentration decreased again to 0.02. At a lower flow $q_{mid+ref}$, breakthrough occurred at 7.8 PV and increased up to a maximum relative concentration of 0.07, which decreased to 0 after 11.4 PV. For linuron, breakthrough only occurred at a flow q_{max} with an increase in relative concentration to 0.03 after 8.92 PV; this concentration decreased to 0 after 10.85 PV. Breakthrough of metamitron occurred at q_{max} and $q_{mid+ref}$ after respectively, 6.8 and 7.3 PV and reached a maximum relative concentration of respectively 0.08 and 0.06. Metamitron in the effluent decreased however to 0 after 10.4 and 8.5 PV for q_{max} and $q_{mid+ref}$ respectively.

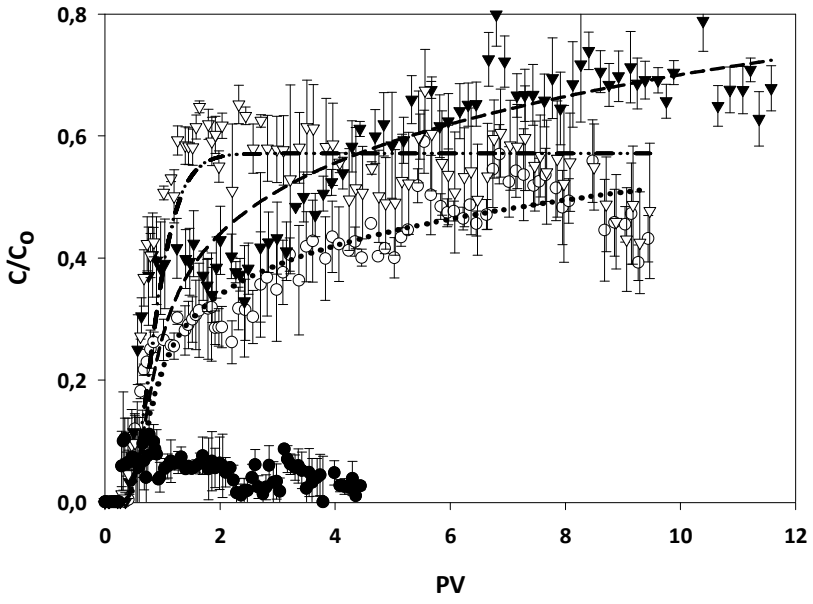

Figure 6.7: Observed and simulated BTCs of bentazone in 4 experimental macrocosm set-ups with variable water flux. Observed BTCs are presented with the following symbols: ● , q_{min}, ○ , q_{mid}; ▼ , q_{max}; ▽ , $q_{mid + ref}$. Simulated BTCs are presented as: ——— , q_{min}; · · · · · · · , q_{mid}; — — , q_{max}; — · · , $q_{mid + ref}$. Relative concentrations are plotted against the number of pore volumes (PV) (error bars are ± standard deviation (p < 0.05) (n=3)).

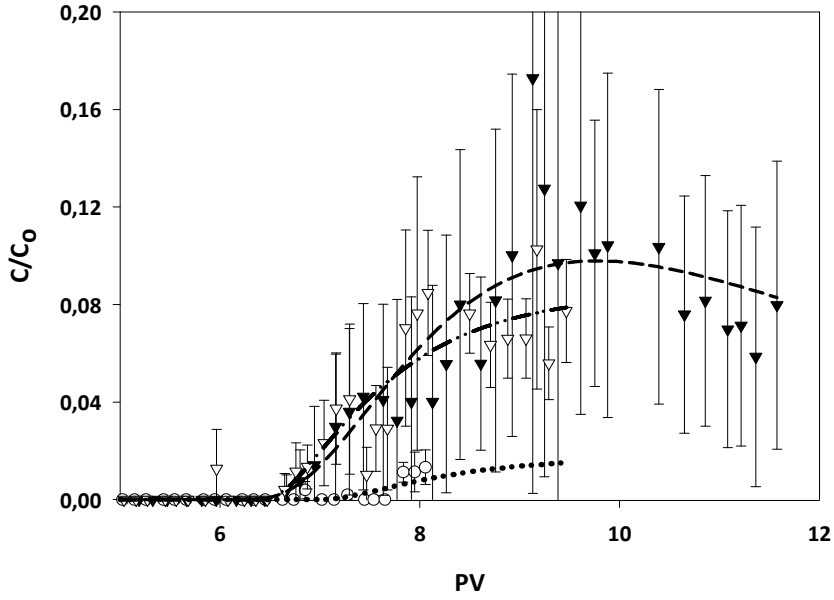

Figure 6.8: Observed and simulated BTCs of metalaxyl in 4 experimental macrocosm set-ups with variable water flux. Observed BTCs are presented with the following symbols: ● , q_{min}, ○ , q_{mid}; ▼ , q_{max}; ▽ , $q_{mid + ref}$. Simulated BTCs are presented as: ——— , q_{min}; · · · · · · · , q_{mid}; — — , q_{max}; — · · , $q_{mid + ref}$. Relative concentrations are plotted against the number of pore volumes (PV) (error bars are ± standard deviation (p < 0.05) (n=3)).

6.4.2.3 Sorption parameters of metalaxyl, bentazone, isoproturon, linuron and metamitron

Transport of isoproturon, linuron and metamitron could not be fitted due to low or non-detectable concentrations in the effluent at all flows. Bentazone and metalaxyl concentrations in the effluent were higher, which made fitting of the breakthrough curve possible (respectively Figure 6.7 and Figure 6.8). The estimated sorption parameters are presented in Table 6.5. Bentazone effluent concentrations were very low at q_{min}, with a removal of the initial concentration of about ± 90%, which made estimation of the sorption parameters at the low flow impossible. For metalaxyl, no detectable concentrations could be measured at q_{min}.

Table 6.5: Predicted sorption parameters (K_f, n), and determination coefficient (R^2) of bentazone and metalaxyl determined with the CDE model with equilibrium sorption in columns submissive to different flow regimes (± 95% confidence interval) (n=3).

		Bentazone	
Flow	K_f [$L\ kg^{-1}$]	***n***	R^2
q_{min}	/	/	/
q_{mid}	$6.19 \cdot 10^{-4} \pm 5.82 \cdot 10^{-4}$	6.02 ± 0.56	0.89
q_{max}	$5.94 \cdot 10^{-4} \pm 5.94 \cdot 10^{-7}$	5.46 ± 0.50	0.87
$q_{mid+ref}$	$6.41 \cdot 10^{-7} \pm 6.03 \cdot 10^{-5}$	2.05 ± 138,12	0.91
		Metalaxyl	
Flow	K_f [$L\ kg^{-1}$]	***n***	R^2
q_{min}	/	/	/
q_{mid}	15.3 ± 1.37	0.49 ± 0.06	0.93
q_{max}	33.1 ± 4.63	0.67 ± 0.14	/
$q_{mid+ref}$	21.4 ± 3.77	0.51 ± 0.06	0.91

Freundlich parameters K_f and n of bentazone are similar to the values obtained in the macrocosms in Chapter 4. These values are however not very meaningful due to their high uncertainty. Nevertheless, limited sorption which could be observed in micro- and macrocosms in Chapter 4 and in the microcosms in 6.4.1.3 also occurred in the macrocosms under variable flow and thus confirms previous findings. Retention of bentazone does not appear to vary between the different flows. Breakthrough appears to occur more or less simultaneously.

The sorption capacity of the organic matrix for metalaxyl in the macrocosms studied in this chapter appeared to be higher than the sorption capacity of mixture 10 in the macrocosms studied in Chapter 4, but is however similar to the values obtained in the microcosms at q_{min}. The higher fraction of coco chips and peat mix could have increased the sorption capacity of

the matrix compared to the matrix used in Chapter 4. Differences in breakthrough could not be observed between q_{max} and $q_{mid+ref}$, which is in accordance with the observations in the microcosms. Breakthrough of metalaxyl at q_{mid} occurs slightly later, However due to the fairly high standard deviations on the data points, differences are small. When the concentration in the effluent decreases, variability between the repetitions increases as the analytical error increases.

6.4.2.4 Degradation of metalaxyl, metamitron, isoproturon, bentazone and linuron

Compared to the microcosms, degradation or the formation of bound residues was drastically higher. A comparison in the removal of the initial concentration obtained in the micro- and macrocosms for the different fluxes, is presented in Table 6.6. It is clear that a higher removal in pesticide concentration is achieved in the macrocosms, presumably due to a longer residence time and lower chemical and hydraulic load. The difference in removal was lowest for linuron, where a high removal was also obtained in the microcosms. Although bentazone was shown to be a pesticide with a very low potential to be retained in the column experiments, a drastic increase in removal could be observed when the chemical and hydraulic load decreased.

In general, the highest removal in effluent concentration could be achieved at the lowest flow q_{min}, with > 90% removal for all pesticides, while the removal varied from 15 to 94% at q_{max}. In addition, it could be stated that the use of pesticide-primed soils reduced the effluent concentration slightly for most pesticides.

Table 6.6: A comparison of the removal of the initial concentration between the micro and macrocosms (removal was calculated as the difference in relative concentration between, the initial concentration and the maximum effluent concentration reached)

Pesticide	Removal (%)							
	Macrocosm				Microcosm			
	q_{min}	q_{mid}	q_{max}	$q_{mid+ref}$	q_{min}	q_{mid}	q_{max}	$q_{mid+ref}$
Bentazone	90.23	37.08	15.43	28.11	0.00	0.00	0.00	0.00
Isoproturon	100.00	100.00	93.01	91.24	48.82	15.32	3.11	26.80
Linuron	100.00	100.00	94.46	100.00	93.42	94.60	92.63	93.14
Metalaxyl	100.00	97.60	85.49	83.36	91.54	51.79	0.00	0.00
Metamitron	100.00	100.00	93.57	92.43	89.29	78.59	67.88	57.17

Similarly to the determination of the sorption parameters, degradation parameters could only be determined for metalaxyl and bentazone and are presented in Table 6.7. For bentazone it could be concluded that the highest degradation occurred in $q_{mid+ref}$. This could however lead to misinterpretations as the fitted BTCs of bentazone did not reach a steady-state yet at q_{mid} and q_{max}. Visually, it could be concluded that there was no significant difference between q_{mid} and $q_{mid+ref}$, while a lower degradation and thus higher steady-state concentration could be observed at q_{max}.

Table 6.7: Estimated first-order degradation coefficient μ_l (h^{-1}) of bentazone and metalaxyl in columns with variable flux (± 95% confidence interval) (n=3)

Pesticide	Flow	μ_l (x 10^{-3}) [h^{-1}]
Bentazone	q_{min}	/
	q_{mid}	$1{,}57 \cdot 10^{-6} \pm 3.96 \cdot 10^{-17}$
	q_{max}	$3{,}13 \cdot 10^{-6} \pm 4.56 \cdot 10^{-17}$
	$q_{mid+ref}$	$1{,}40 \cdot 10^{-3} \pm 8.27 \cdot 10^{-5}$
Metalaxyl	q_{min}	/
	q_{mid}	$1.26 \cdot 10^{-2} \pm 4.57 \cdot 10^{-4}$
	q_{max}	/
	$q_{mid+ref}$	$7.05 \cdot 10^{-3} \pm 2.30 \cdot 10^{-4}$

Degradation of metalaxyl at the highest flow could again be described using the Monod model as the effluent concentration appeared to decrease slightly. From Figure 6.8 and Table 6.7, it could be concluded that degradation at q_{mid} was significantly higher than at $q_{mid+ref}$. Consequently, the incorporation of metalaxyl-primed soil increased dissipation of metalaxyl, which is in accordance with the results obtained in Chapter 5 and with the observations in the microcosm.

6.4.2.5 Mass balance of pesticides

The mass balance of bentazone, isoproturon, linuron, metalaxyl and metamitron in the macrocosms is presented in Figure 6.9. The pattern of the obtained mass balance is slightly different than what could be observed in the microcosms.

In the macrocosms, the amount of **bentazone** leached varied from 3 to 41% and the amount retained varied from 9 to 13%, which is respectively much lower and higher than in the microcosms. A decrease in hydraulic and chemical load could have enhanced dissipation. The highest dissipation could be observed at a flow q_{min}, with 86% dissipated. A decrease in flow in the macrocosms drastically reduces the leaching of the most mobile pesticide studied. The dissipation of bentazone in the macrocosms studied in Chapter 4 was similar to the observed results and is in accordance with the results of Boivin *et al.*[217], who observed a formation of 65% bound residues of bentazone in a clay soil.

Isoproturon which did not or hardly leach out of the macrocosms at the different flows, appeared to be very little dissipated. The majority (98-99%) of the applied isoproturon was present in the MeOH extractable fraction. This means that no non-extractable residues are formed or that degradation of isoproturon in the organic matrix did not occur. Dissipation of isoproturon in the microcosms was much higher than in the macrocosms, which is in contrast to what is expected as the longer residence time and a lower chemical and hydraulic load in the macrocosm mostly increased dissipation of the pesticide.

In line with the previous observations, leaching of **linuron** was negligible. The MeOH extractable fraction (20-79%) was however higher than what was observed in 4.4.2.5 and in the microcosms, with the lowest amount of linuron sorbed in the macrocosms where a high flow was applied. A higher dissipation could be observed at q_{max} (80% at q_{max} compared to 21% at q_{min}). This might be contributed to a faster increase linuron degrading community due to a higher linuron input at q_{max} (cfr. 6.4.2.6).

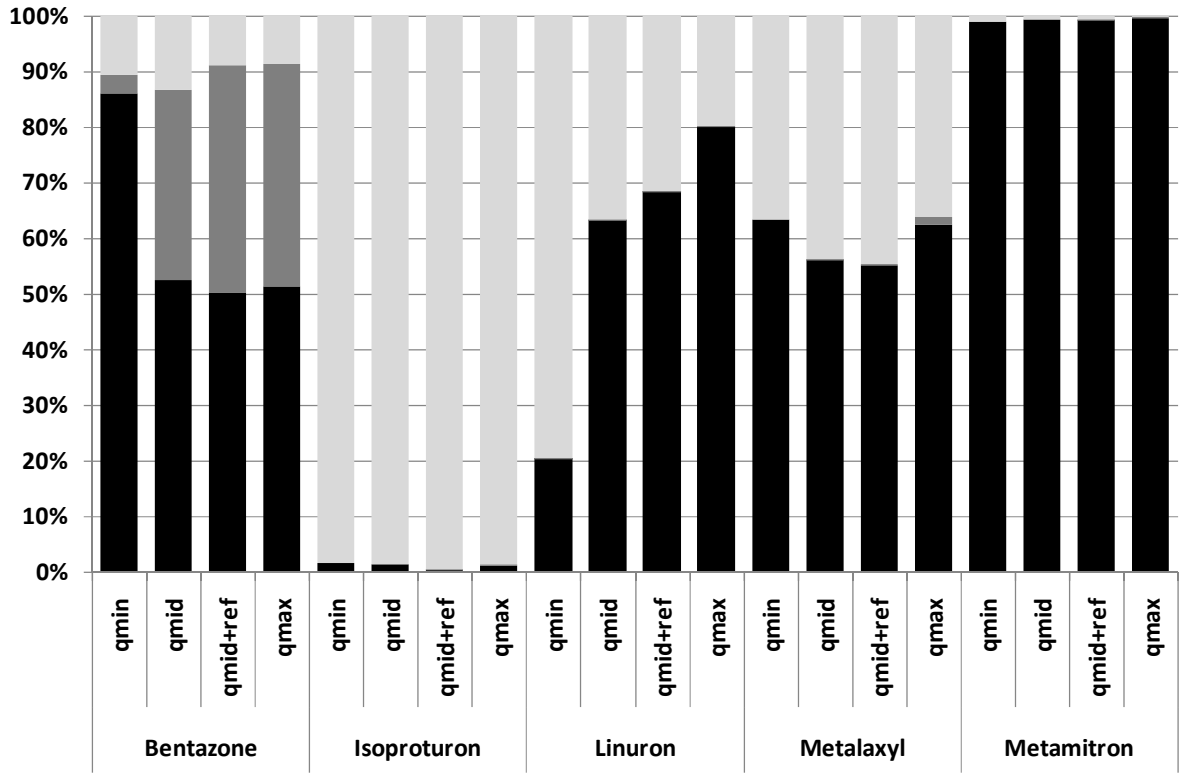

Figure 6.9: Mass balance of bentazone, isoproturon, linuron, metalaxyl, and metamitron in macrocosms with variable flux. ■ *= % dissipated or bound residues;* ■ *= % MeOH extractable fraction* ■ *= % leached.*

Degradation or the formation of non-extractable residues of **metalaxyl** varied from 55-63%, which indicates that differences between the different fluxes were small. This is in contrast to the results found in the microcosms, where a lower degradation could be observed at q_{max} and $q_{mid+ref}$. However, the longer residence time could provide more time for the metalaxyl degrading community to develop, which leads to similar levels in community size at all flows. The amount of MeOH extractable fraction is much higher in the macrocosms than in the microcosms, which is off course reflected in a lower metalaxyl concentration in the effluent. Due to the larger volume and the lower chemical load in the macrocosms, more sorption sites will be available, which could increase the retained metalaxyl.

Metamitron, which was easily dissipated in the microcosms, is dissipated to an even higher extent in the macrocosms (99-100%), independently of the flow or the presence of pesticide-primed soil. Thus a decrease in chemical and hydraulic load, increases the probability for metamitron biodegradation. The fraction of sorbed metamitron to the matrix was very low (0.1-1.1%) and is in accordance with the results observed in the microcosms.

6.4.2.6 Batch degradation experiments

Batch degradation experiments were carried out with organic matrix from the macrocosms as inoculum. Substratum was sampled at the top of the matrix, as the highest microbial activity was previously observed in this layer for most pesticides (Chapter 4). Sampling was done after 12, 60 and 123 days of pesticide input in the column, to check the evolution in degradation in time. The first-order degradation rate μ and the lag time of linuron, metalaxyl, metamitron and isoproturon were determined from the degradation curves. The degradation rate μ was not graphically presented as differences in degradation rate were small and not significant. μ varied between $7.95 \cdot 10^{-3}$ to $1.01 \cdot 10^{-2}$ h^{-1} for pesticides which showed degradation. The evolution in lag time is presented in Figure 6.10. Data of bentazone were not present in both graphs as no degradation occurred during the time frame studied, which is in accordance to the results in 4.4.3.7. As no microbial degradation was observed in the batch experiments, the dissipation observed in the BTCs of bentazone is probably mainly caused by an increase in the non-extractable residues. Nonetheless, results from bentazone mineralization experiments performed by Sniegowski, K. (personal communication) with matrix samples from a similar experiment in presence and absence of a liquid medium, showed that 15% of ^{14}C-labelled bentazon was converted to CO_2 within 65 days in case no medium was added compared to less than 1% when a liquid medium was added. This indicates that although no degradation was detected in the batch experiment, bentazon can be degraded slowly in the matrix itself.

The lag time was considered as the time before exponential degradation occurred. During this lag time, the pesticide concentration either remained constant at the initial value or decreased linearly to a certain value after which exponential decrease occurred. During this linear phase, it could be possible that other populations are responsible for degradation. Linear degradation could indicate co-metabolic degradation (*i.e.* pesticide does not serve as an energy source for the microorganisms), while the exponential phase points to metabolic degradation (*i.e.* pesticide can serve as a substrate for growth).

Lag time or acclimation period can be defined as the delayed response or adaptation to a change in the environment. The lag time is highly variable and might depend on the type of pollutant, exposure and environmental conditions, and microbial community structure. Adaptation of the microbiota can be the result of several mechanisms, including proliferation of the pesticide degrading community, induction of pesticide degrading enzymes, diauxie and the appearance of new genotypes[218]. However as the observed lag times varied from 0 to 44 days, the short lag times will rather be the result of enzyme induction[187] or the presence of a relatively high pesticide degrading biomass[136]. The lag time provides an impression of the pesticide degrading community dynamics in the macrocosm in time. The obtained lag times can not always be extrapolated to lag times present in the column experiments, as conditions in batch are not always comparable to the situation in the column. For example, differences in aeration and mixing might exist. Moreover, in batch experiments a decrease in substrate concentration will occur compared with the constant influent substrate concentrations in column experiments[219].

The highest lag time for linuron degradation could be observed at q_{min} after 10 days of pesticide input in the column. This lag time decreased with increasing flow. This indicates that a higher amount of linuron input provides more nutrients to the community without

being toxic. No significant differences could be observed in lag time between columns inoculated with pesticide-primed soil or reference soil, which indicates that a linuron degrading community was already present in the reference soil or that it also gained the ability to degrade linuron, which is in accordance with the observations of Sniegowski et al.[192]. Finally, a significant decrease in lag time could be observed with time. After 60 and 123 days, no acclimation period was present and degradation started immediately. This decrease in lag time could confirm the observation in 6.4.2.5, where a higher dissipation of linuron was observed at q_{max} which could be attributed to a higher linuron degrading community caused by a higher linuron input at q_{max}.

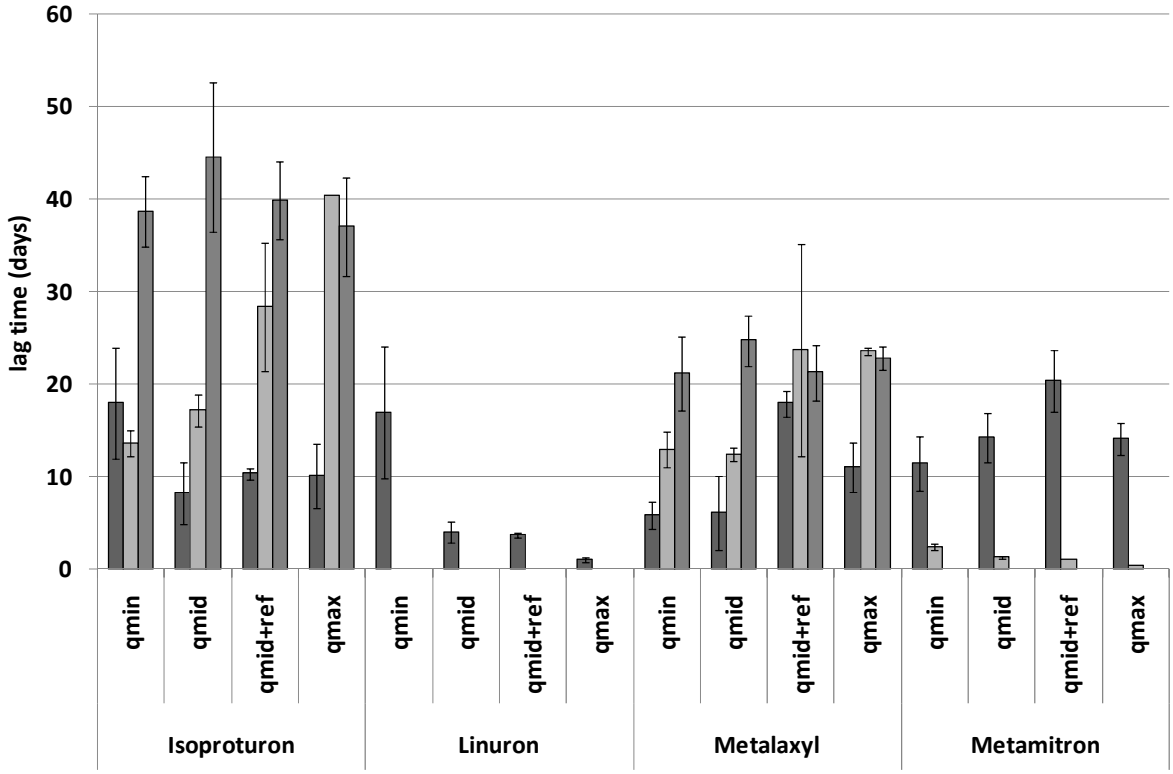

Figure 6.10: Evolution of the lag time (days) in time of linuron, metalaxyl, metamitron and isoproturon in the matrix sampled from macrocosms with variable flux and with or without the incorporation of pesticide-primed soil. (■ = after 10 days; □ = after 60 days; ■ = 123 days) (error bars are ± standard deviation (p < 0.05) (n=3))

For isoproturon and metalaxyl, an increase in lag time could be observed after 60 and 123 days. This could indicate that the pesticide degrading community was present, but decreased the longer metalaxyl and isoproturon were applied to the organic matrix. From the mass balance (6.4.2.5) it was observed that for isoproturon and to a lesser extent also for metalaxyl, a larger fraction of the applied pesticide was still present as extractable fraction. This might indicate that the pesticides were available and not strongly bound to the organic matrix. These pesticides probably accumulated in time. This increase in isoproturon and metalaxyl concentration in the matrix could have reached toxic levels for the pesticide degrading community in the matrix, with as a result, an increase in lag time in time. A

decrease in the pesticide degrading microbial community was also observed by Prado and Airoldi [220], where higher 2,4-D concentrations decreased the biodegradation.

The increase in lag time did not come to expression in the breakthrough curves, because degradation or retention was probably still high enough to prevent or minimize isoproturon and metalaxyl to breakthrough. The presence of metalaxyl in the effluent at $q_{mid+ref}$ and q_{max} (Figure 6.8) could be the result of a lower initial degradation due to the higher lag time of metalaxyl degradation at $q_{mid+ref}$ and q_{max} after 10 days and the increase in lag time in time. The presence of a lag time could also be observed in the BTCs, where delayed degradation occurred in the columns receiving metalaxyl at a flow q_{max}. For metalaxyl, it could be stated that inoculation of the matrix with the reference soil led to a lower lag time in the beginning of the experiment. However, as the initial community in the metalaxyl-primed soil also decreased, lag times were not significantly different anymore after 123 days. In addition, no significant differences in lag time could be observed between the different fluxes after 123 days, which could indicate that the size of the metalaxyl degrading community was similar for all flows. This also confirms the results of the mass balance where dissipation of metalaxyl was similar at all flows.

Just as for linuron, a significant decrease in lag time could be observed after 60 days of metamitron application to the column. The largest decrease could be found in the column receiving the highest flow. Thus a higher amount of metamitron applied to the column, probably stimulates the proliferation of the microorganisms. The inoculation of pesticide-primed soil to the column improved degradation slightly at the start of the experiment. However after 60 days of application, lag time of the column with reference soil was similar to the columns with pesticide-primed soil.

6.4.2.7 Conclusion

Sorption of the studied pesticides in the microcosms was significantly influenced by the applied flux on top of the columns for pesticides which are fairly mobile (isoproturon, metamitron and metalaxyl). A high flux significantly decreased retention of the pesticides, due to the sorption process which is time dependent at high water fluxes. However, for very mobile pesticides, such as bentazone, the influence of a lower flux did not significantly increase retention as breakthrough of this pesticides already occurs very fast, even at a low water flux. The same accounts for more immobile pesticides such as linuron, where an increase in flux did not appear to provoke an early breakthrough of this pesticide in the time frame studied.

Degradation of the fairly mobile pesticides was also significantly influenced by the flux. Pesticides which are strongly retained (linuron) or which have a high leaching potential (bentazone) appear to be less submissive to the influence of water flux on degradation. The opportunity time, that may be required for maximum degradation, decreases as a result of faster transport of a pesticide through the organic matrix at a high flux. Moreover, a higher input of pesticides can lead to the presence of toxic levels to the pesticide degrading microbiota or a higher flow might lead to a distribution of the pesticide degrading biomass.

On a microcosm scale, it could be stated that an increase of the flow applied to a biopurification system is not beneficial for the retention and degradation of pesticides.

Moreover, the addition of pesticide primed soil did not always appear to enhance degradation of the studied pesticides significantly, with the exception of metalaxyl where a higher degradation in the presence of pesticide-primed soil could be observed.

Compared to the microcosms, very limited breakthrough occurred in the macrocosms for metamitron, isoproturon, linuron and metamitron. No detectable concentrations could be measured in the effluent of columns submissive to q_{min} and q_{mid} and a reduction of the initial concentration of > 91% could be measured at the flows $q_{mid+ref}$ and q_{max}. A fit to the breakthrough curves at these low concentrations was not possible, hence no sorption and degradation parameters could be obtained for isoproturon, linuron, metamitron and metalaxyl at q_{min} and q_{mid}. The influence of flow on bentazone concentration in the effluent was significant compared to the other pesticides. The lowest bentazone concentration in the effluent could be obtained at the flow q_{min}.

Batch degradation experiments were carried out with time to have an idea on the pesticide degrading community dynamics by the determination of the lag time or adaptation period. No degradation of bentazone could be observed, which indicates that most of the bentazone dissipation observed in the columns will be caused by an increase in bound residues or by populations or species not able to grow under the conditions applied in batch degradation experiments. The lag time of metamitron and linuron decreased drastically in time for all flows, indicating a growth in the pesticide degrading community. This is in contrast to isoproturon and metalaxyl, where an increase in lag time could be observed in time for all flows. From the batch degradation experiments, it could be concluded that the influence of flow on the lag time was minimal, certainly after 123 days of pesticide application and that the inoculation of the pesticide-primed soil had a little surplus value on degradation compared to the reference soil. The inoculation of pesticide-primed soil could be beneficial in the removal of some pesticides during the start-up phase, as a lower lag time could be observed for metalaxyl and metamitron in the macrocosms with pesticide-primed soil.

A mass balance was made on the input, output and rest fraction of all pesticides in the substratum in the macrocosms. A higher dissipation of bentazone could be observed in the macrocosms compared to the microcosms, certainly at q_{min}. Isoproturon appeared to be sorbed to the organic matrix as the MeOH extractable fraction approached almost 100% of the initial concentration for all flows. Linuron was dissipated to a lesser extend at a lower flow. Thus, a higher pesticide input, stimulated degradation of this pesticide. From the mass balance, it could be concluded that the flow had little influence on the distribution of metalaxyl over the different phases. Finally, metamitron showed a higher dissipation compared to the microcosms for all flows.

Chapter 7: The influence of small and large scale composting/incubation on the dissipation of pesticide residues in a biopurification matrix

7.1 Abstract

The efficiency of the biopurification system will start to decrease as the matrix mineralizes slowly in time and nutrients become depleted. As a result a decay in biomass will probably occur and should the matrix be replaced. The spent matrix might however still contain residues of pesticides. Hence, treatment of this matrix is essential. In this study it was opted to use composting or incubation as an effective and environmentally friendly treatment strategy. Small and large scale composting/incubation trials were set-up to treat linuron, bentazone, metalaxyl and bifenthrin in a contaminated matrix. Large scale composting, performed in an industrial composting facility with tunnel composting, resulted in a decrease in concentration of metalaxyl, linuron and bentazone. Degradation of bifenthrin, the most persistent pesticide, was very limited. In the small scale incubation process, a decrease in concentration was visible for bifenthrin, metalaxyl and bentazone. A removal in extractable pesticide concentration does, however, not always indicate degradation but could also be attributed to the formation of non-extractable residues. It could be concluded that industrial and small scale incubation did reduce the concentration of some pesticides during the time frame studied, although little removal was obtained for the persistent pesticide bifenthrin in the industrial composting process and for linuron in the barrel incubation.

7.2 Introduction

As shown in previous chapters, pesticides are quite efficiently retained and degraded in the organic matrix of the biopurification system. As this matrix mineralizes slowly in time, nutrients will start to become depleted and thus a decay in biomass will probably occur. At that moment, it has been suggested that the efficiency of the biopurification system will start to decrease and that the matrix should be replaced. The spent matrix might however still contain residues of pesticides.

Up till now, no consensus has been reached on the treatment of this matrix after use. In some regions or countries (*e.g.* Flanders) it is still considered as hazardous waste and thus needs special remediation treatments. Dispersal, collection, removal, land fill disposal and incineration are possible remediation techniques for a contaminated matrix. However, these techniques are expensive and simply dilute, or sequester the contaminants, or transfer them to another environmental medium.

Composting can be considered as a more effective and environmentally friendly strategy, since it can result in the partial or complete biotransformation of pesticides to microbial biomass and stable, innocuous end-products. Composting is an aerobic process that relies on the actions of microorganisms to degrade organic materials. The composting process can be divided into four major biological stages in relation to temperature: mesophilic, thermophilic, cooling and maturation. With these changes in temperature, there are related changes in the structure and composition of the microbial community. With an increase in the respiratory activity, there is an increase in temperature resulting in microbial community shifts towards thermophilic microorganisms. It is at these higher temperatures (45-65°C) that most of the decomposition and biomass formation takes place[221-223]. In the third phase, there is a cooling down effect due to the decrease in microbial activity as most of the direct utilizable organic carbon has been removed, resulting in an increase in community density and diversity of mesophilic microorganisms[221-223].

Up till now very little research is present on the fate of pesticides in the biopurification matrix after use. Torstensson[52] evaluated the residues of 26 pesticides during one year. He observed that the concentrations of 13 out of 26 pesticides were below the detection limit, while only small residues remained during storage in a contained place. The underlying processes of pesticide dissipation were however not elaborated. To comply with the regulations of waste regulation, residues must be restricted to an absolute minimum[53].

A number of studies have been performed on pesticide degradation during composting. Kupper *et al.*[224] demonstrated that composting of 28 pesticides was successful in full scale composting plants, with a dissipation rate of more than 50% for more than two-thirds of the studied pesticides after 112 days of treatment. Michel *et al.*[225-227] reported results from several composting studies concerning the fate of diazinon, 2,4-D and pendimethalin. After 50 days of composting, respectively 50%, 11% and 13% of 2,4-D, diazinon and pendimethalin was mineralized. Kuo and Regan[228] observed a decrease of 97.5% of carbaryl in spent mushroom substratum after 24 days. Dooley *et al.*[229] conducted experiments to investigate the dissipation of dicamba in a mixture of cattle manure and wood chips, 98% dicamba was degraded after 53 days.

Composting can be performed on several scales, including small scale composting in a compost bin and full scale industrial composting. Essentially the same biological processes are involved in both scales of composting, although the techniques used, the management of the process and several other factors must be taken into account. Small scale composting is mainly performed in plastic bins or barrels or in wooden constructions which enable to process wastes generated in the kitchen or the garden of individual households. Alternatively, wastes can also be composted centralized on an industrial scale. The latter process is highly controlled and often consists of windrows which are continuously aerated.

The aim of this study was to study the fate and behavior of pesticides in two different composting processes: barrel and industrial composting/incubation. The evolution of the concentrations in time of the residues of four pesticides was followed-up. The selected pesticides varied in their persistence and mobility in order to be able to gain insight in the fate of pesticides with variable transport characteristics.

7.3 Materials and methods

7.3.1 Pesticide properties and chemicals

The studied pesticides were metalaxyl, bentazone are non-persistent, mobile pesticides. Bifenthrin (2-methyl-1,1-biphenyl-3-y1)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropane-carboxylate) (K_{oc} = 225844 *L* kg^{-1}, $t_{1/2}$= 30 *d*) is a persistent, immobile pesticide. Linuron is a non-persistent, immobile pesticide. Methanol, acetonitrile, hexane, acetone and water were of A.R. grade (VWR, Leuven, Belgium).

7.3.2 Biopurification matrix

The contaminated matrix originated from a biopurification system (biofilter)(Figure 1.11) which was treated with 18 different pesticides from which the four previously mentioned pesticides were selected. The biofilter consisted of 4 units, filled with a mixture of organic substratums (70% peat mix, 20% straw, 5% soil and 5% coco chips).

7.3.3 Large scale or industrial composting

In the industrial composting facility DDS-Verko (Dendermonde, Belgium) source separated organic household wastes (also called vegetable, fruit and garden wastes or biowastes) are shredded and subsequently composted in GICOM tunnels (l x w x h: 22 *m* x 5.5 *m* x 4.5 *m*; GICOM, Biddinghuizen, the Netherlands) (Figure 7.1).

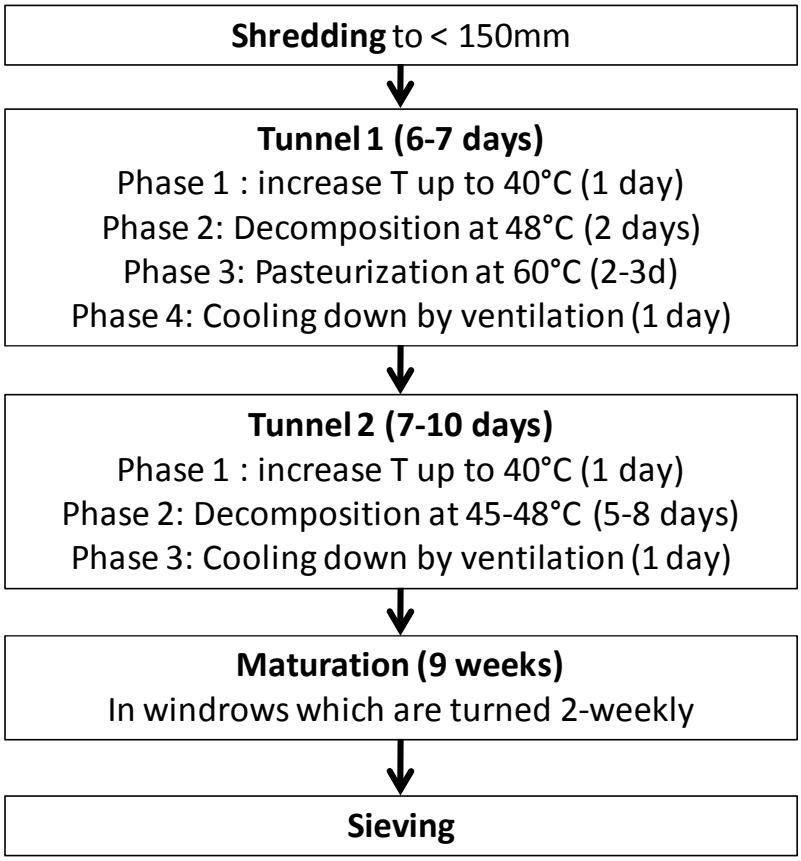

Figure 7.1: Overview of the management of biowaste in the industrial composting process.

The tunnels are continuously aerated through forced ventilation. The temperatures of the compost heaps in the tunnel are on-line monitored with four probes which are linked to a central computer which in turn regulates the intensity of the aeration. Above the composting pile there is a low under pressure of about 2-5 *Pa*. The composting pile is also moistened with sealing sprayers to prevent dehydration of the composting materials due to the forced aeration and self-heating of the composting feedstocks. Consequently, in these tunnels there is a good distribution of oxygen, temperature and moisture. Dependent on the composition of the initial material and the desired end quality, the initial stages of the composting process take intensively place in the tunnels during a period of 2 to 4 weeks. After the tunnel composting the materials are further composted and finally matured in an enclosed hall without forced aeration. Here, weekly or two-weekly turnings with a front leader support sufficient aeration. The whole course of the composting process takes about 10 weeks after which the compost is sieved (15 mm).

At the start of the experiment, pesticide-contaminated biofilter matrix was divided over 9 nylon bags (mesh: 100 *µm*), each containing 3.2 *kg* of the substratum. Six of these nylon bags were equally divided over and incorporated in 3 HDPE (High Density Poly Ethylene) potato bags (60 *cm* x 105 *cm*) containing shredded biowaste feedstock. An individual temperature data logger El-USB-1 (Lascar Electronics, Salisbury, United Kingdom) was added to each potato bag. Then, the 3 potato bags were buried in the compost heap at a depth of about 40-45 *cm* from the top (total height of the pile: 2.8 *m*). Besides, three nylon bags with contaminated matrix and three control samples of surrounding material (shredded

biowaste) were analyzed immediately at the start of the experiment. After 7 days, the potato bags were collected from the first tunnel and buried on the same depth in the second tunnel (total height of the pile: 2.5 *m*). After 15 days, a nylon bag was removed from each potato bag together with some surrounding material and both were analyzed. The three remaining potato bags, each containing nylon bags with surrounding material, were then transferred to the enclosed hall for further composting and maturation. In this phase, the compost was stored in a windrow (l x b x h: 10 *m* x 4.5 *m* x 2.5 *m*) and was turned weekly until 58 days after the start of the experiment. The experiment ended after 76 days, when the last 3 bags containing contaminated matrix and surrounding material were analyzed for remaining residues.

7.3.4 Small scale incubation

Six compost barrels (89x80 *cm*, 280 *L*, GRAF, Germany) were filled with 180 *kg* of contaminated matrix and stored in a greenhouse at constant temperature. A temperature datalogger (EL-USB Lite, Lascar, Switzerland), which registers temperature every hour, was inserted in the middle of the barrel. A sample of the matrix was collected from each barrel to determine the initial pesticide concentration. After 22 and 46 days, three of the six barrels were emptied and 10 *kg* of dried cow manure was added, the other barrels were turned. After this, samples were taken after 47, 84, 115, 161, 229 days and analyzed.

7.3.5 Pesticide analysis

Extraction of metalaxyl, bentazone and linuron was carried out as described in Chapter 4 (4.3.4). Analysis of these pesticides with HPLC-DAD was described in Chapter 3 (3.3.3). Extraction of bifenthrin was carried out on 50 ± 0.001 *g* organic matrix of which the dry matter content was determined gravimetrically after drying at 105°C during 24 hours. 200 *mL* of 1:1 hexane/acetone (v/v) was added to the organic matrix and shaken during 1 *h* at 150 *rpm*. The liquid phase was separated from the solid phase with a Buchner filter and transferred to a separation funnel to which 200 *mL* of distilled water was added together with 20 *mL* of saturated NaCl solution. After shaking by hand for about 90 *s*, the water layer was removed and this procedure with water and NaCl solution was repeated. The organic phase, hexane, was filtered over $NaSO_4$ to remove any water still present. The remaining solution was evaporated until dryness with a rotavapor. Bifenthrin was redissolved in 1:1 hexane/acetone (*v/v*) and injected in the gas chromatograph with mass spectrometer (GC-MS).

Gas chromatographic analysis was performed on an Agilent 6890 GC equipped with a 5973 inert MSD. An HP-5MS capillary column (30 *m* x 0.25 *mm* i.d., 0.25 *µm* film thickness, J&W Scientific, USA) was used. The oven program was as follows: 70°C during 2 *min* as initial temperature, a 25°C/*min* ramp to 150°C, a 3°C/*min* ramp to 200°C, an 8°C/*min* ramp to 280°C, and 10 *min* at 280°C. A split/splitless injector was used in the splitless mode. The carrier gas was helium with a constant column head pressure of 137 *kPa*. The injector and transfer line temperatures were 280 and 250°C, respectively. One microliter of sample was injected. Mass detection was performed in the single-ion monitoring (SIM) mode. The selected ions used for detection and quantification were 165 *m/z* and 166 *m/z*, with a

retention time of 28.84 *min*. The ions were selected from the fragments with the highest m/z values and strongest signals.

7.4 Results and discussion

7.4.1 Environmental conditions

The temperatures in the nylon bags and in the barrels which were continuously measured with data loggers during the course of the composting experiment are presented in Figure 7.2A and B. The temperatures of the piles in the tunnels were also on-line registered with a central data logging system of DDS-Verko. Both loggings reveal similar temperature patterns (data not shown).

In Figure 7.2A, it can be observed that an elevated temperature (> 45°C) is maintained during the whole industrial composting process. Fluctuations are caused by the removal of the samples before turning of the compost heap and subsequential reincorporation of the samples. The thermophilic phase which starts after about 12 h, reaches a maximum temperature of 80°C during the secondary composting. During the cooling down phase, temperatures decrease after ± 50 *d* and reach a temperature of about 45°C. The compost starts to mature from this stage on. These temperature profiles are in accordance with former observations and are indicative of effective humification[222,230].

Temperatures in the barrels are lower (Figure 7.2B), with a maximum temperature of 42°C. The lower temperatures are caused by the smaller volume of compost compared to the heap in industrial composting, which makes heat losses more important. Moreover, lower temperatures are also the result of a lack of nutrients as the waste feedstock in this experiment is only composed of biofilter substratum. This substratum is already mineralized to a large extent during its use in the biopurification system. Consequently, further decomposition will be limited. Nevertheless, elevated temperatures can be observed at two points in time for these barrels with addition of dried cow manure. The peaks of the temperature curve coincide with the moment were cow manure was added. Nitrogen is a critical element for microbial growth, N limitation may decrease degradation[222]. Thus the addition of manure may stimulate microbial metabolic activity and consequently increase temperature. A thermophilic phase was not really observed. Thus, real composting did not occur, which means that elevated temperatures were not reached. As no fresh material was added and as no elevated temperatures were reached, this process will further be indicated as ‘incubation’ instead of ‘composting’.

Moistening was only executed during industrial composting in tunnels. Moistening was limited in the first tunnel as the incoming waste feedstocks were already relativity wet (38%). Excessive moistening was however done in the second tunnel to prevent drying out of the composting materials. During secondary composting in the enclosed hall no further moistening was performed. Tunnels 1 and 2 were irrigated with respectively 165 $L\ m^{-2}$ and 661 $L\ m^{-2}$ water.

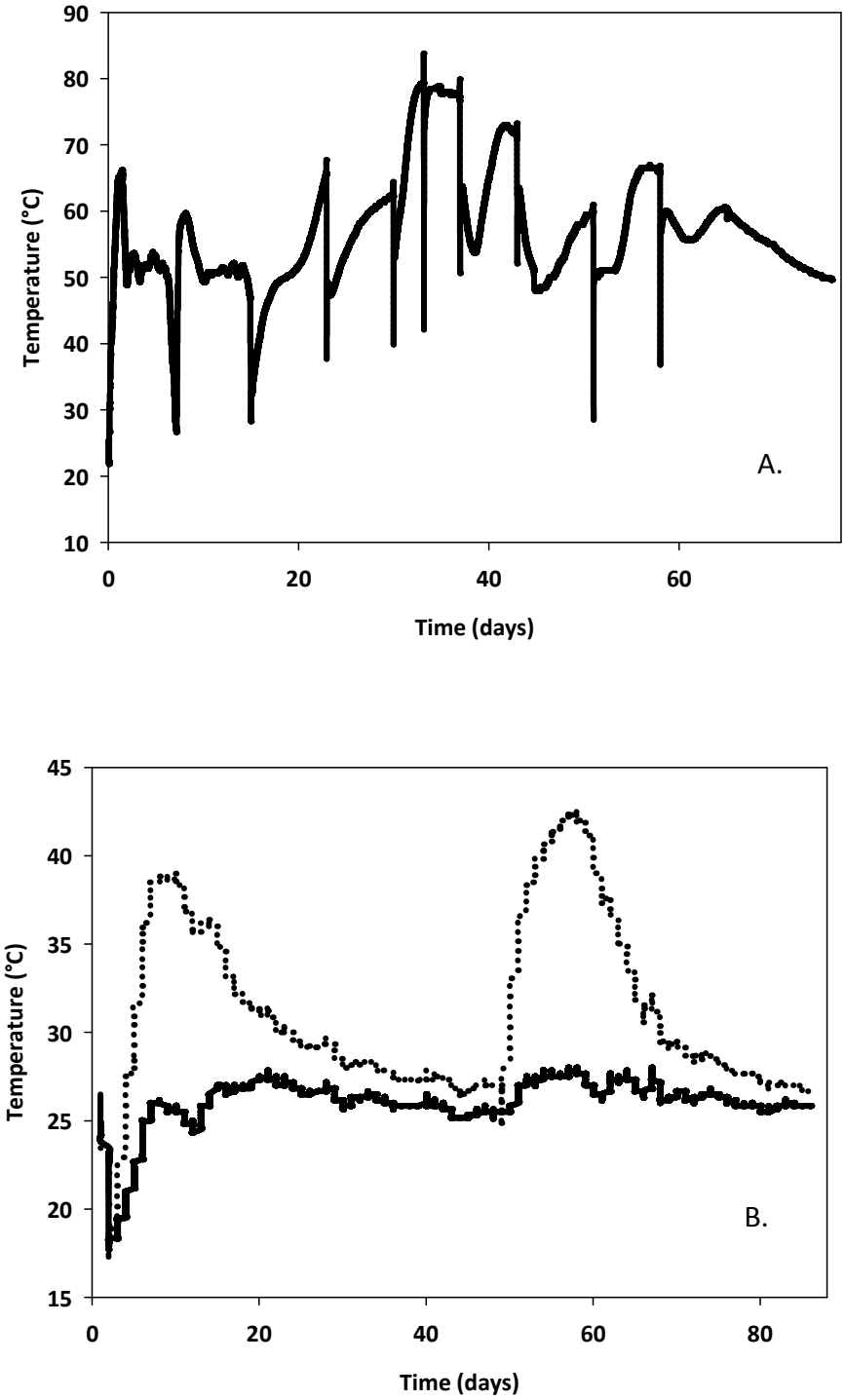

Figure 7.2: Temperature evolution during the industrial and barrel composting/incubation process. A. Industrial composting, B. Barrel incubation (━━ =contaminated matrix ; ····· = contaminated matrix with additional cow manure)(error bars are ± standard deviation (P < 0.05) (n=3)).

7.4.2 Physicochemical characteristics

The physicochemical characteristics of the contaminated matrix and the surrounding material in the industrial composting process are presented in Table 7.1 at the start of the composting process and at the end after maturation. An increase in dry matter content could be expected. This increase presumably occurs during maturation as no irrigation was applied. The decrease in organic carbon is the result of the mineralization of the organic material during composting. An increase in available phosphate, potassium, calcium and sodium could also be observed, which is in accordance with the results of Jackson and Line[231], Zucconi and De Bertoldi[232] and Sellami *et al.*[233]. A slight increase occurred in the total N content, which could be caused by degradation of proteins in the biomass during the thermophilic phase or resulted from the loss of dry weight as carbon dioxide and water evaporate[233]. The C/N ratio of the contaminated matrix was just outside the optimal range for rapid and entire humification, which is between 25 and 35[222,234]. This high C/N ratio was attributed to the fairly low total N content. The C/N ratio of the surrounding material was lower and located in the optimal range. The C/N ratio of both fractions decreased after composting due to the decrease in organic carbon and due to the rather stable N content. During the industrial composting of the contaminated matrix, an increase in pH from 6 to 9 could be observed. This increase in pH is caused by the degradation of acids which are formed in the beginning of the composting process and by ammonification[222,235]. These factors can however only partially explain this remarkable pH increase, which is most probably the result of leaching of organic compounds and salts from the surrounding material. The pH of the surrounding material remained more constant. Finally, the electrical conductivity (EC) increased significantly during composting due to degradation of volatile solids and to an increase in the amount of water-soluble salts on a total solids basis[235].

Table 7.1: Physico-chemical characteristics of the contaminated matrix and the surrounding material at the start and end (77 days) of the industrial composting process.

	Contaminated matrix		Surrounding material	
	start	**end**	**start**	**end**
Dry matter (%)	32.7	37.4	39.4	48.4
Organic carbon (%)	54.2	45.0	61.2	51.3
P_2O_5 ($mg\ L^{-1}$)	7.70	47.9	224	491
K_2O ($mg\ L^{-1}$)	205	2210	1090	2120
CaO ($mg\ L^{-1}$)	1000	1120	1690	3120
MgO ($mg\ L^{-1}$)	2620	273	196	325
Na_2O ($mg\ L^{-1}$)	44.8	410	221	376
Total N (% on OM)	0.25	0.38	0.51	0.61
C/N	39.33	24.56	26.25	22.59
pH	6.4	9.1	9.0	9.2
EC ($\mu S\ cm^{-1}$)	183	1310	655	1270

The physicochemical characteristics of the cow manure and the contaminated matrix at the start and the end of barrel incubation are presented in Table 7.2. A distinction was made between the barrels with only the contaminated matrix and barrels where dried cow manure was added.

Similar to the industrial composting process, an increase in the dry matter content and a decrease in the organic carbon content could be observed. Furthermore, increases in the available phosphate, potassium, calcium and sodium could also be observed, which is in agreement with the results of the change in mineral composition occurring during the industrial composting process. The increase in minerals of the contaminated matrix where no cow manure was added was smaller as the change in the industrial composting. The increase in the industrial composting could, as mentioned, be the result of leaching of extractable minerals of the surrounding material. The increase of the minerals in the matrix where cow manure was added, was higher. This is in accordance with the results of Sellami *et al.*[233] where the incorporation of poultry manure increased the minerals present in the compost. The increase in total N content of the contaminated matrix with cow manure is attributed to the incorporation of the N rich manure. The slight increase in total N content of the contaminated matrix was not as high as during the industrial composting process. Due to the lower temperatures in the barrel incubation, degradation of proteins of the biomass will be limited. Similar to the industrial composting, a decrease in the C/N ratio of the contaminated matrix could be observed in all barrels. A higher decrease in C/N ratio of the contaminated matrix with cow manure is attributed to the incorporation of cow manure. The fact that the pH of the contaminated matrix remains stable during incubation, confirms the hypothesis that organic compounds and salts from the surrounding materials entered the contaminated matrix in the industrial composting process. Finally, the EC decreased slightly in the contaminated matrix in the barrels, while it increased in the barrels where cow manure was added. The increase in EC was also observed by Sanchez-Monedero *et al.*[236] and Gil *et al.*[237]. They stated that EC increases because of the increased concentration of nutrients, such as nitrate. A decrease in EC in the contaminated matrix could be caused by leaching of organic compounds or salts. This is however unlikely as no water was added to the compost bin.

Table 7.2: Physico-chemical characteristics of the added cow manure and the contaminated matrix in the barrels at the start and end (229 days) of the small scale incubation process. Physico-chemical characteristics of barrels with and without the addition of cow manure were separated.

	Cow manure	Start	Without cow manure	With cow manure
			After 229 days	After 229 days
Dry matter (%)	86.7	32.7	40.2	43.3
Organic carbon (%)	37.6	54.2	43.9	45.1
P_2O_5 ($mg\ l^{-1}$)	98700	7.70	7.92	448
K_2O ($mg\ l^{-1}$)	113700	205	289	1120
CaO ($mg\ l^{-1}$)	18000	1000	1210	1870
MgO ($mg\ l^{-1}$)	49200	2620	336	686
Na_2O ($mg\ l^{-1}$)	14100	44.8	65.3	147
Total N (% on OM)	6.6	0.25	0.28	0.37
C/N	15.2	39.33	35.12	29.28
pH	6.9	6.4	6.8	6.3
EC ($\mu S\ cm^{-1}$)	510	183	133	1190

7.4.3 Pesticide residues

7.4.3.1 Industrial composting

As mentioned in the section Materials and Methods, samples were taken at the start of the experiment, followed by sampling after tunnel composting (after 15 days) and finally at the end after maturation (after 76 days). Figure 7.3 shows the relative residues of bifenthrin, bentazone, metalaxyl and linuron in function of time in the contaminated matrix and in the surrounding compost.

Pesticides are subjected to a series of physicochemical and biological processes during composting, such as sorption, leaching, volatilization, biotic and abiotic transformation or mineralization, which determine the extent of their dissipation. Sorption can lead to the formation of bound residues, which are non-extractable and thus cannot be captured by chemical analysis[224]. Michel *et al.*[238] have found that almost 50% of ^{14}C-ring labeled 2,4-D was complexed with humic acids or the humin fraction of compost derived from yard trimmings. The dissipation rates found in the current study are driven by a combination of the processes listed above but the experimental design did not allow to assign the relevant processes. Therefore the formation of bound residues, volatilization and leaching should also be considered. Volatilization and leaching of pesticides during composting are mainly determined by the chemical structure and the composting process. Volatilization is mainly limited by sorption and physical barriers within the compost, however, air movement, induced by ventilation, may promote volatilization. Leaching, which is also highly pesticide dependent, can also be induced during the composting process. Too excessive moistening in the full scale composting process may increase leaching of mobile pesticides towards the water collector (buffer tank) of the composting facility.

It could be observed that the behaviour of the pesticides with varying physicochemical characteristics varies quite significantly. A significant decrease (75%) in the bentazone concentration is visible after 15 days but remains constant during the maturation phase (Figure 7.3). Bentazon is a very mobile pesticide and will therefore be very submissive to leaching out of the nylon bag. Due to irrigation of the heap, water flows might be created inside the heap which could transport the very water soluble bentazon molecules out of the bag into the surrounding material. However, only 17% of the initial bentazone concentration could be detected in the surrounding material, which indicates that bentazone is either degraded or leached further down the heap, of which the latter is most probable. During the secondary composting and maturation phase little degradation of bentazone could be observed. The dissipation observed between the initial phase and the maturation phase could also be caused by degradation by white rot fungi such as *Phanerochaete chrysosporium*[239], however increased temperatures and high pH could also have reduced the activity of these pesticide degraders[240]. According to Vyas *et al.*[241] optimum temperatures for the production of manganese and lignin peroxidase are lower than the optimal temperature for growth of *Phanerochaete chrysosporium* (39-40°C) and thus temperatures in industrial composting could be too high. Thus leaching beyond the surrounding material appears to be the most plausible dissipation pathway. Moreover, as bentazone is an ionic pesticide, an increase in pH results in a higher leaching potential of this pesticide[242].

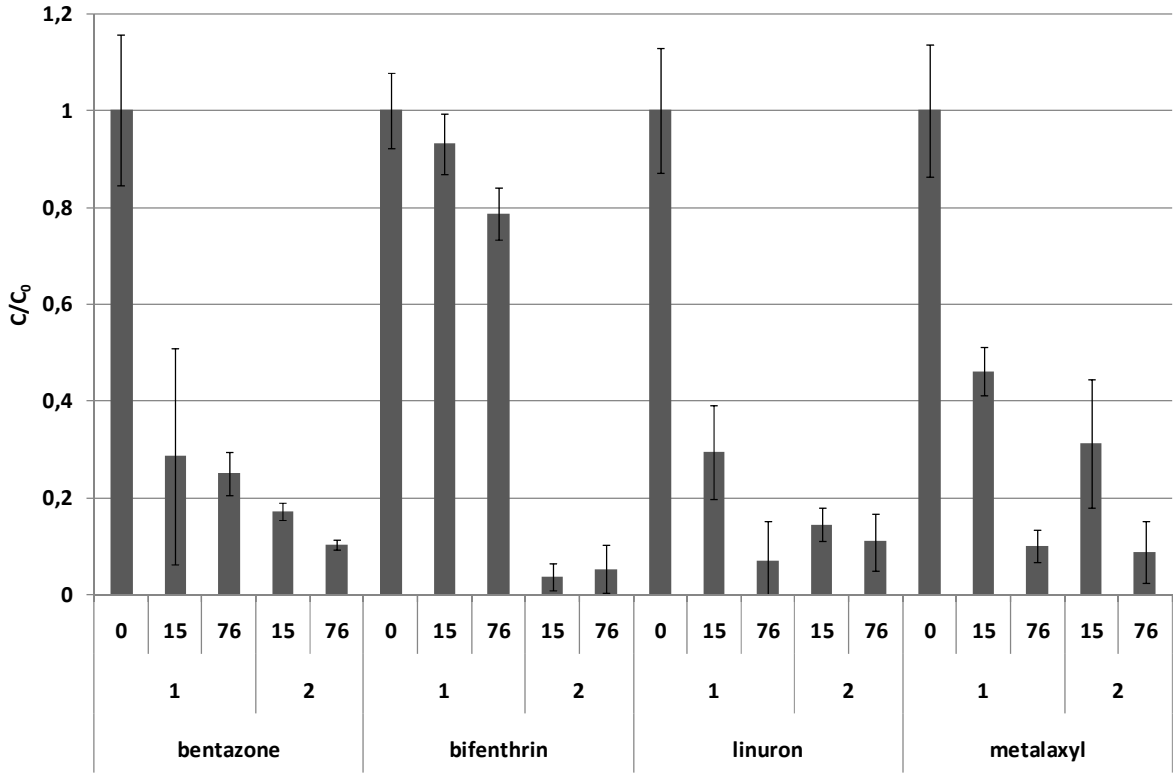

Figure 7.3: Relative pesticide residues in the contaminated matrix (1) and surrounding compost (2) composted in an industrial composting plant at the start (day 0), after tunnel composting (day 15) and after maturation (day 76) (error bars are ± standard deviation (P < 0.05) (n=3)).

Bifenthrin, the most persistent and immobile pesticide in the studied range, is hardly degraded during the experimental time frame (Figure 7.3). During tunnel composting no significant degradation occurred, while during maturation bifenthrin residues were 20% lower than the initial concentration. This removal could be caused by degradation or by the formation of bound residues. Previous studies by Crossan and Kennedy[243] and Baskaran *et al.*[244] showed that bifenthrin was very stable over a year. Therefore the main pathway for dissipation will be the formation of bound residues as bifenthrin has a very high K_{oc} value. The low mobility of bifenthrin also explains the very low concentrations in the surrounding material, thus migration was limited.

Linuron, a non-persistent and immobile pesticide, showed little leaching out of the nylon bag during irrigation (Figure 7.3). A significant dissipation of 70% could be observed during tunnel composting. 14% of the linuron dissipation after tunnel composting is caused by migration during irrigation, thus still a significant degradation of linuron could be observed. Finally, 93% of linuron was dissipated after 76 days. As no irrigation is performed in this final stage, leaching will be limited and thus, dissipation will mainly be caused by degradation or the formation of bound residues. As favourable conditions (C/N ratio, moisture content, pH, etc.) for linuron biodegradation are present and as this is the main route for the removal of linuron, biodegradation is the most likely pathway. Complete mineralization of linuron has been reported by Dejonghe *et al.*[138] and Sörensen *et al.*[245]. Moreover, Rodriguez-Cruz *et al.*[246], observed an enhanced microbiological degradation in soil with the amendment of city refuse compost.

Finally, metalaxyl which is less mobile than bentazone, shows a dissipation of 54% after tunnel composting. And a dissipation of 90% of the initial concentration is achieved after maturation (Figure 7.3). As metalaxyl is fairly mobile, 31% of the initial amount migrated to the surrounding material. However, dissipation of metalaxyl also appears to occur in the contaminated matrix and the surrounding material after tunnel composting where no irrigation is applied, indicating that degradation could have occurred during the maturation phase. Degradation of metalaxyl in biopurification systems was already observed for several times[49,67,247].

7.4.3.2 Small scale incubation

Pesticides residues of bifenthrin, linuron, metalaxyl and bentazone in the contaminated matrix were also followed-up during 229 days of barrel incubation. The evolution in time of relative pesticide residues are presented in Figure 7.4. A distinction in the barrels was made between barrels with only contaminated matrix and barrels where cow manure was added to the contaminated matrix. As the contaminated matrix was already strongly mineralized during the use of the biopurification system, biodegradation of the organic material by microorganisms will be limited and thus microbial activity will be lower compared to the active composting of fresh substratums. Low biodegradation of the substratum is also reflected in the relatively low temperatures during incubation. To test the influence of the addition of exogenous nutrients to 3 out of 6 barrels, dried cow manure was added at two different points in time. In Chapter 4, it could be observed that the incorporation of dried cow manure in the matrix of a macro scale biopurification system stimulated the biodegradation of metalaxyl, isoproturon and bentazone, which is in accordance with the results of Pigeon *et al.*[248]. As mentioned above, this addition resulted in an increase in temperature of about 15-20°C.

The bifenthrin concentration in the contaminated matrix decreased from resp. 48% and 51% in barrels with and without dried cow manure after 229 days of barrel incubation (Figure 7.4). The dissipation of bifenthrin could be caused by degradation or the formation of bound residues. However, as mentioned before, the experimental set-up did not allow to differentiate between these two processes. A sterile control was set-up by autoclaving the biowaste five times. However, after a few months there were traces of fungal contamination in the sterile control, which made the sterile control ineffective.

The concentration of linuron, the second most immobile pesticide of the studied molecules did not decrease in time during barrel incubation (Figure 7.4), which is in contrast with the results observed during the industrial composting process and with the results obtained in Chapter 4. Moreover, no differences could be observed in the fate of linuron in barrels with or without added dried cow manure. Differences in degradation between the industrial and the small scale composting/incubation experiment could be caused by the more favourable higher pH and higher amount of extractable potassium in the contaminated matrix after industrial composting (Table 7.1 and Table 7.2). This is in accordance with the results of Rasmussen *et al.*[249], who found a positive correlation between the water extractable potassium content, pH and linuron mineralization in Danish soils. Moreover, higher temperatures present in the industrial composting facility could have enhanced pesticide degradation[187,250,251].

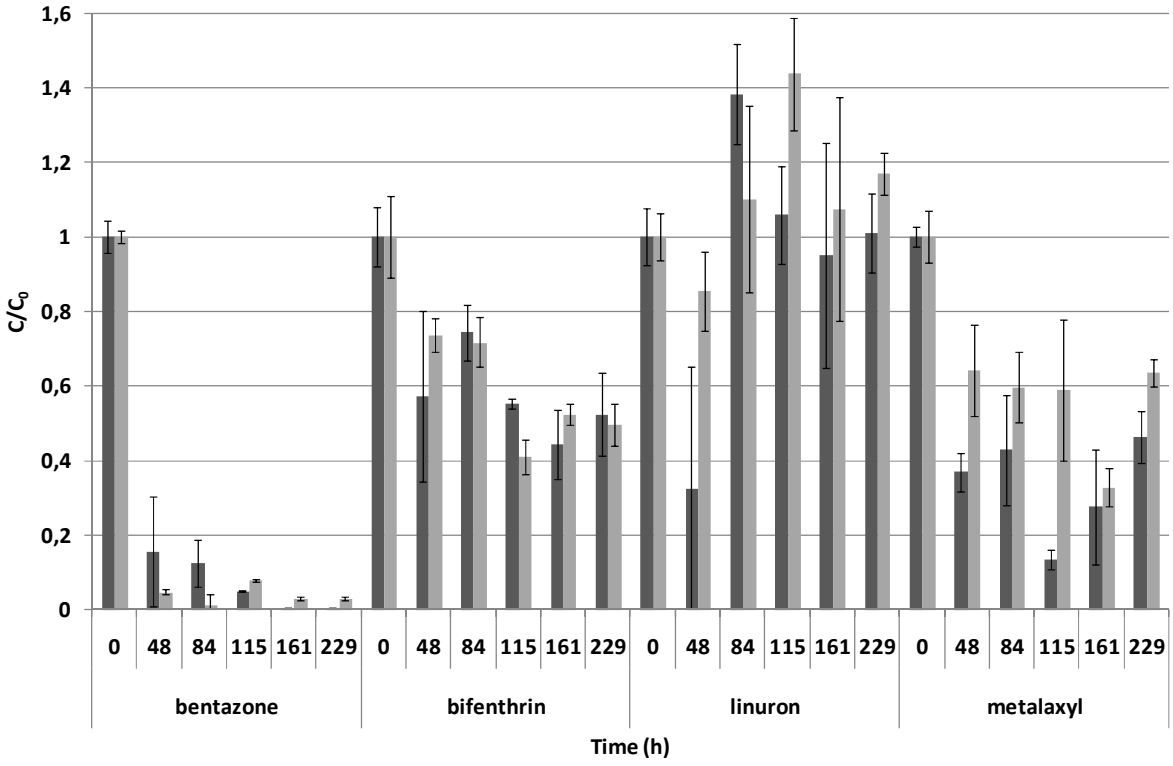

Figure 7.4: Evolution of the relative concentration of bifenthrin, linuron, metalaxyl and bentazone in the contaminated matrix composted in compost barrels during 7 months (= no addition of cow manure; = with addition of cow manure) (error bars are ± standard deviation (P < 0.05) (n=3)).

After 7.5 months 54 and 37% of metalaxyl was degraded in respectively the barrels with and without the addition of cow manure (Figure 7.4). This confirms previous results (Chapter 4) were metalaxyl was fairly easily degraded in micro and macro scale column experiments. In the column experiments it was however observed that the addition of cow manure stimulated the degradation of metalaxyl. The slightly higher degradation of metalaxyl in the barrels without added nitrogen, could be caused by the negative effect of nitrogen on the microbial degradation. As a lower amount of cow manure was added in the column experiments (5%) compared to the small scale barrel incubation (±11%), the higher amount of added cow manure could have inhibited the degradation of metalaxyl due to a decrease of soil microbial biomass[252] or due to a metabolic shift in the soil microbiota[253,254].

Dissipation of bentazone was very high with respectively 99% and 97% in the barrels with and without the addition of dried cow manure. Dissipation of bentazone was also very high in the industrial composting process. However, irrigation in this process could have caused leaching of bentazone in the compost heap. As there was no water flow in the barrels, leaching of bentazone can not explain the decrease in concentration in the small scale experiment. The dissipation could be attributed to the formation of bound residues which is in accordance to the work of Boivin *et al.*[217] where 85% of the initial applied bentazone was non-extractable after 160 days and with the results of Gaston *et al.*[255], where the amount of unextractable C^{14} labeled bentazone increased with time. As bentazone is an ionic pesticide

and as the pH is lower in the barrel incubation process compared to industrial composting (± 6.5 compared to 9.1), sorption of bentazone could have increased[242]. The current observations are also in accordance with the results from Chapter 6. A high dissipation of bentazone could be observed in the macrocosms at the lowest flux. Hence, it could be hypothesized that a very low or no water flow enhances the dissipation of this very mobile pesticide drastically. The half-life value of bentazone in water is extremely high ($t_{1/2}$ = 716 *d*) compared to the half-life value in soil ($t_{1/2}$ = 14 *d*), hence the lower the water content, the higher the dissipation.

7.5 CONCLUSION

A comparison was made between industrial and barrel composting/incubation. In the industrial composting process a decrease in the concentration of metalaxyl, linuron and bentazone could be observed. Degradation of bifenthrin, the most persistent pesticide, was very limited. However, as the contaminated matrix was packed in a nylon bag and was thus not really blended with the composting feedstocks (nevertheless, some exchange was possible via the gaseous and the liquid phase), the experiment should mainly be considered as an incubation test. Incorporation of the material into the compost heap could decrease the pesticide concentration, but this decrease could than also be the result of a dilution of the pesticides. Moreover, this incorporation could lead to contamination of the end product. In the small scale incubation process, a decrease in concentration was visible for bifenthrin, metalaxyl and bentazone. As no sterile control was present, a distinction could not be made between degradation and the formation of non-extractable residues. If pesticides are present at high concentration as non-extractable residues, there is still a problem for the discharge of the spent matrix. The addition of dried cow manure did not appear to enhance the degradation of the pesticides present. As the contaminated matrix was already degraded for a big extent, composting of the material was limited and therefore did not result in elevated temperatures normally occurring during a composting process. For that reason, a suggestion for future research could be the amendment of fresh material to initiate the composting process. Finally, it could be concluded that industrial and small scale incubation did reduce the concentration of some pesticides during the time frame studied, however little removal was obtained for the persistent pesticide bifenthrin. Thus additional treatments still appears to be necessary in order to discharge the material *e.g.* by incorporation in the field.

Chapter 8: Conclusions and future perspectives

Unsatisfactory management of pesticides can give rise to residues in surface- and groundwater, which is a major-environmental issue in Europe[4-6]. One source of contamination is the use of pesticides in agriculture. A large fraction of this contamination is generated on-farm and is called point-source pollution. This point source pollution can significantly be reduced by applying agricultural best management practices and routines. However, some releases may still occur which makes it difficult to come up to the quality standard of pesticides in drinking water which are specified by the EU Directive (98/83/EEC). To come up to these standards, additional technologies are required to reduce direct pesticide releases. A possible approach is the use of biopurification systems to capture and treat contaminated water. Biopurification systems exist under various forms such as, biobed, phytobac©, biofilter, biomassbed, etc. All these systems have proven to be highly efficient in treating pesticide contaminated water, with a removal of 95% of the pesticide concentration in the water. However, up till now these systems all functioned as a black box, which means that the processes taking place inside the system are not well characterized. To be able to optimize and control the biopurification system, further research was necessary.

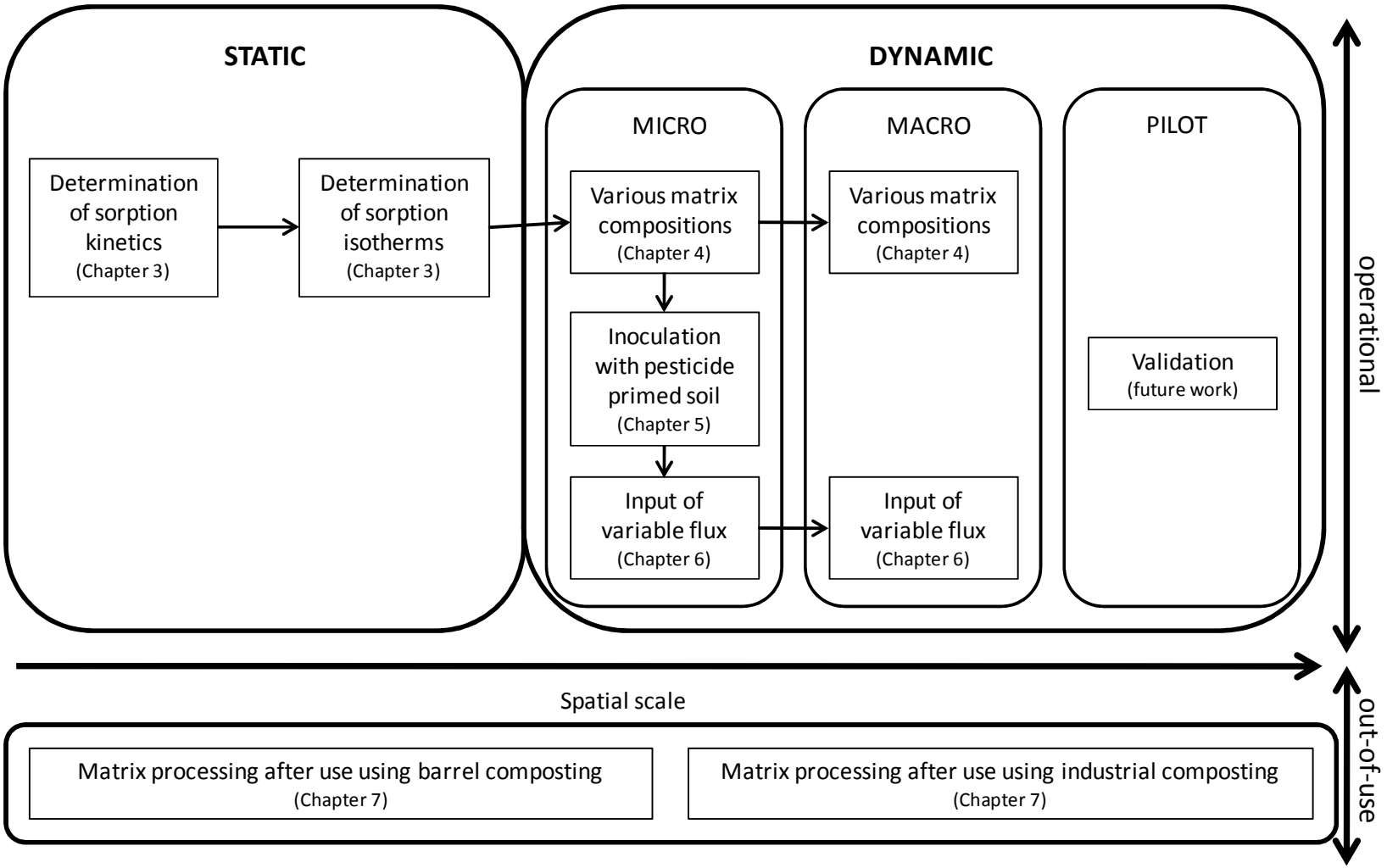

Figure 8.1: Schematic representation of the thesis

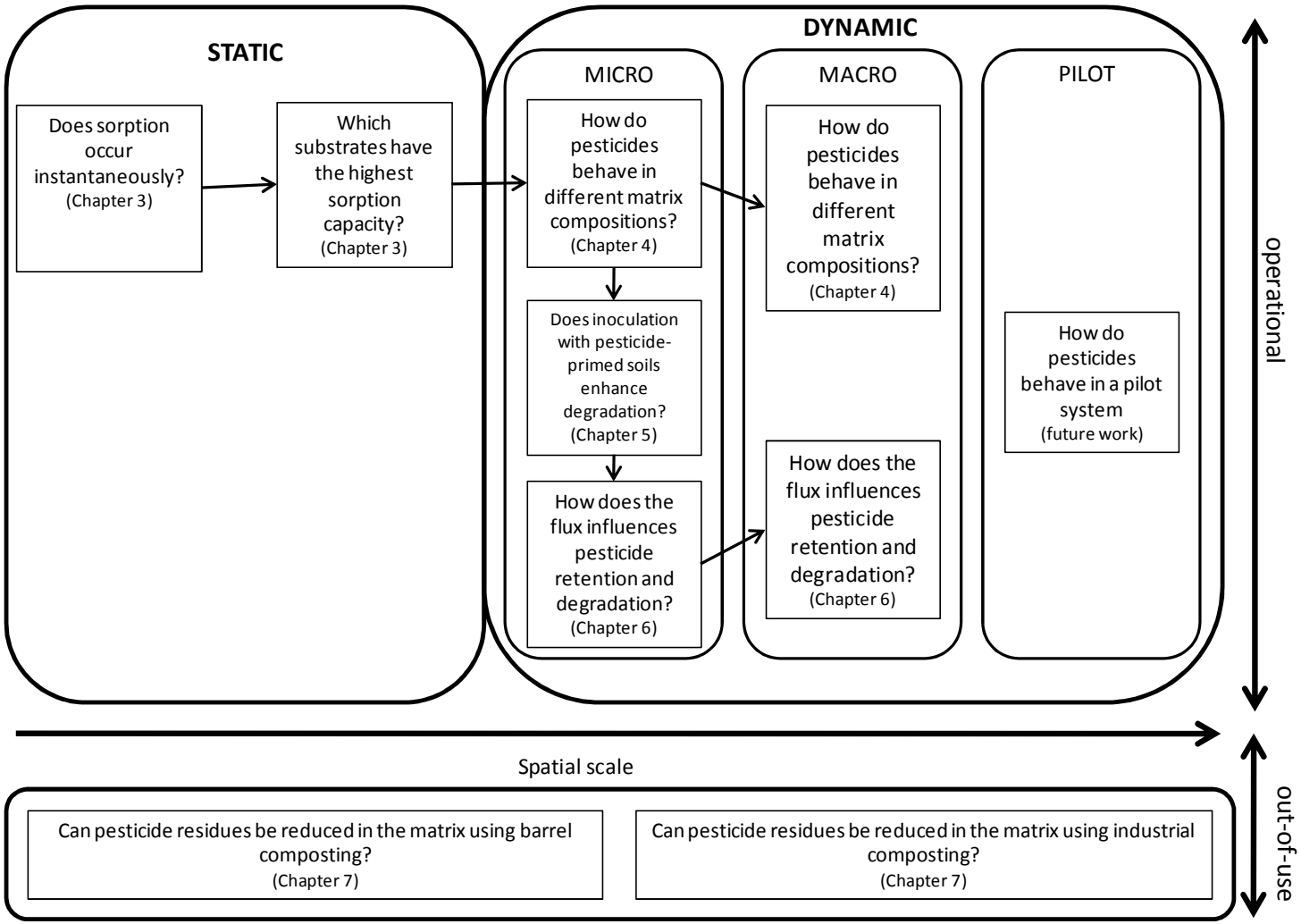

Figure 8.2: Questions posed to define the objectives

The proposed objectives presented in Chapter 2 together with a comparison between the physico-chemical treatments and biopurification systems, will be elaborated in this chapter. This overview provides at the same time a summary of the main aims and findings of this work. In addition, although this research has advanced the understanding of the ongoing process in a biopurification system, several aspects still deserve further experimental investigation. Therefore, several perspectives for future research will also be presented.

The schematic representation of the thesis is presented in Figure 8.1. The major part of the experimental work is performed on the characterization of the processes occurring inside the biopurification system when it is operational. The first section of this work was performed in static conditions or in batch, which means that transport of pesticides was not taken into account compared to the second section (dynamic) where transport was included. In the second section, a series of displacement or column experiments were carried out to validate the results obtained in batch experiments (Chapter 3) and to better understand the functioning of the biopurification system (Chapter 4, 5 and 6). The second part focuses on the processing of the contaminated organic matrix, when the organic matrix is worn out and not efficient anymore, hence when it is taken out-of use. The different compartments presented in Figure 8.1 will be discussed separately as questions which were posed to define the objectives (Figure 8.2).

8.1 Does the biopurification system has a surplus value compared to physico-chemical treatments?

Before the objectives of the thesis are elaborated, a reflection is made to Chapter 1. To answer the above posed question, a comparison between the two types of treatments systems (biopurification and physico-chemical (e.g. Sentinel)) was made in Table 8.1.

Table 8.1: Advantages and disadvantages of the biopurification system and the Sentinel (physico-chemical treatment)

Biopurification system	
Advantages	**Disadvantages**
• Simple and practical • Can easily be self-constructed • Relatively low construction price • A phytobac can treat large amounts of waste water • User-friendly • Flexible (containers of a biofilter can easily be replaced or added) • Possibility of complete mineralization of the pesticide • Low running cost • limited maintenance required during operation	• No legal framework yet for the use of these systems in Flanders (Belgium) • A biofilter can not treat large volumes of waste water • Biological methods are susceptible to toxic compounds that inactivate the waste degrading microorganisms • Possibility of incomplete metabolism (formation of metabolites) • Compound specificity
Sentinel	
Advantages	**Disadvantages**
• Treatment of large quantities of waste water • Treatment of a wide range of pesticides • Water can be re-used	• Expensive • Operator training needed • High running costs (chemicals, maintenance) • Rest fraction

Finally, a comparison was made between the costs per year of biological and chemical treatment with a biofilter, fytobac© and the Sentinel. A biofilter costs about 46€ per year over 15 years, a phytobac costs about 60€ per year also over 15 years, while a sentinel will cost 3808€ per year over 10 years (Peter Jaeken, Phytofar). Hence, considering the overall performance and economical outline of the costs, biopurification systems seem to be a valuable alternative for physico-chemical treatment systems. However, due to the fact that it is a biological system submissive to environmental factors, a constant level of performance can not be assured compared to the physico-chemical treatment systems.

8.2 Study under static conditions

8.2.1 Does sorption occur instantaneously to organic substratums?

Ideally, sorption should occur fast so that leaching through the biopurification system is reduced. However, a number of sorption studies have been published which show considerable variation in the time necessary for the establishment of equilibrium[95,110,140,256,257]. Rate-limited sorption can have serious implications for modeling solute transport. If predictive modeling of pesticide leaching through a biopurification system is performed with the assumption of equilibrium sorption, leaching will be underestimated, which could have implications on the management of the system.

Therefore, sorption of five pesticides (metalaxyl, lenacil, linuron, isoproturon and isoxaben) was studied on seven organic substratums (peat mix, garden waste compost, straw, sandy loam soil, cow manure, coconut chips and willow chopping) in Chapter 3. The first-order kinetic model was fitted to the observed pesticide concentrations in time, resulting in an estimated kinetic rate constant α. Strongly sorbing pesticides, such as isoxaben (K_{oc}= 862 $L\ kg^{-1}$) and linuron (K_{oc}= 410 $L\ kg^{-1}$) showed a high first-order kinetic rate constant α, which indicated the existence of a positive correlation between the distribution coefficient K_d and the kinetic constant α. No relation could however be found between the organic matter content of the substratums and the rate of sorption. Structural differences (specific surface, particle size, etc.) between the substratums could play a major role in the sorption kinetics as e.g. dense material can hinder the diffusion of pesticides into the substratum, which could result in a decrease in the kinetic constant α. Future characterization of the pesticide-substratum interaction should be performed to assess the major factors determining rate-limited sorption of pesticides on the organic material.

To study the effect of the obtained kinetic constant on transport in a biopurification system, simulations were carried out using HYDRUS-1D[105]. Differences in optimized α values could significantly influence the leaching of certain pesticides through biopurification systems, as low rate constants cause a faster leaching and lower degradation, which results into a decrease in efficiency of the system. On the other hand, high α values, representing a fast sorption process, results in transport of the pesticide which is almost identical to the transport process of pesticides where equilibrium sorption is assumed. The influence of the rate constant α on the leaching of a pesticide is thus highly dependent on the mobility of the pesticide. Leaching of pesticides with a high mobility is more liable to changes in the kinetic rate constant and thus to nonequilibrium sorption.

8.2.2 Which organic substratums have the highest sorption capacity for which pesticides?

The sorption capacity of the various organic substratums was studied in the second part of Chapter 3. The functioning of the biopurification system depends mainly on the composition and type of organic material used, as this is crucial for the retention of pesticides, as well as for the amount and activity of microorganisms responsible for degradation of pesticides[40]. The first reported organic matrix used, consisted of peat, straw and topsoil[34]. Alternative

substratums such as compost, coco chips, willow chopping and cow manure were also studied. Gaining insight into the sorptive behavior of pesticides on these substratums can improve retention of pesticides in the biopurification system.

The use of organic material in the prevention of contamination of ground and surface water is not only important in the treatment of point source pollution, but also for diffuse pollution. The pollution by drift (*i.e.* is the movement of airborne spray droplets, vapours, or dust particles away from a target area) can be reduced by incorporating highly sorptive organic material in the buffer zone (*e.g.* peat) to prevent leaching of pesticides to the ground and surface water.

Pesticides with varying mobility were tested on seven different substratums with a diverse range in organic carbon content. To quantify the sorption of the pesticide on the substratum, different sorption models were applied to the obtained sorption isotherms. The Freundlich equation appeared to be the best model to describe sorption. After quantification, substratums could be classified in order of increasing sorption capacity: sandy loam soil < willow chopping, cow manure < straw, coco chips, compost < peat mix. From this ranking and with the function of each substratum in mind, it could be advisable to use peat mix since it is a nutrient source for micro organisms, a substratum with a high water holding capacity and a substratum which is most efficient in retaining pesticides. The use of straw or coco chips should also be included in the matrix as these substratums serve as structure elements. The difference between the two substratums is the available carbon, straw is easily mineralized in contrast to coco chips which could therefore be more sustainable. Willow chopping is also a structure element but is however less suitable for use due to its low sorption capacity. Finally sandy loam soil, with its very low sorptive capacity, can not be excluded as this substratum serves as the major source of possible pesticide degrading micro organisms. The quantity of soil could however be decreased to improve retention. Cow manure with an average sorption capacity is of less importance as this substratum can never be added in large amounts as this would decrease the C/N ratio drastically, which would be pernicious for biodegradation. Hence, the function of the substratum should be the first criteria in the selection of the substratums, followed by its sorption capacity. The sorption capacity of the substratums was positively correlated with the organic carbon content, CaO content and the cation exchange capacity. These parameters can also be accounted for in the selection of the substratums.

Pesticides are hardly treated as a single substance, but are mostly applied in combination with other pesticides. Preliminary tests were performed to test competitive sorption of pesticides. A lower sorption of metalaxyl could be observed in combination with bentazon, linuron and isoproturon. This phenomenon can decrease the efficiency of the system if combinations of antagonistic pesticides are used. However, further research should be performed to fully understand the influence of competitive sorption on the leaching of pesticides in biopurification systems. For example, it could be possible that pesticides can be classified in groups based on their chemical composition and that two groups are antagonistic in their sorptive behavior in the biopurification system. In practice, this could lead to guidelines where certain groups of pesticides can not be treated at the same time.

Pesticides in the field are mostly applied in a formulation. This formulation consists of the active ingredient which actually controls the pest and adjuvants, which are broadly defined

as any substance other than water, without significant pesticide properties, which enhance or is intended to enhance the effectiveness of a pesticide. They include a diverse range of chemical compounds such as petroleum and vegetable oils, solvents, and surfactants. From a regulatory perspective adjuvants are generally considered to be inert, and their influence on pesticide fate and transport processes has largely been ignored. However, adjuvants may significantly enhance or decrease sorption of the pesticides on organic material. In a preliminary study, where sorption of formulated and non-formulated bentazone and isoproturon were compared, it could be observed that the formulation or presence of adjuvants did not have a significant influence on the sorption of isoproturon and bentazon. However, as formulations can vary widely across pesticides, further research of the additives on the leaching of pesticides should be investigated.

Finally, a preliminary test was performed to test whether the sorption coefficient K_d was additive i.e. if a mixture consists of different substratums, the sum of the sorption coefficients K_d obtained on the single substratums equals the sorption coefficient determined on the mixture of substratums. This hypothesis could be confirmed for four pesticides.

8.3 Study under dynamic conditions

8.3.1 How do pesticides behave in different matrix compositions in a biopurification system?

To answer this question profoundly, the experimental set-up changed from static to dynamic. Dynamic means that transport of pesticides was also accounted for and is performed in column displacement experiments. This experimental set-up allows to study both degradation and sorption processes at the same time.

This study, described in Chapter 4, focused on the fate of four pesticides, i.e. linuron, bentazone, metalaxyl and isoproturon on a small (micro) and large (macro) scale. Large scale experiments were performed in barrels to validate the results obtained in the microcosms and to approach a real-life biopurification system. Experiments performed in microcosms tested five different substratum compositions, while in the macrocosms, ten different variations were tested.

The one-dimensional transport model HYDRUS-1D was used to identify and quantify solute transport and hydraulic parameters, and was thus used descriptive rather than predictive. The solute transport and hydraulic parameters were estimated using inverse modeling techniques. Transport was described using the convective-dispersive equation (CDE) with first-order degradation or Monod kinetics (which have been implemented in HDYRUS-1D), the latter to describe the possible occurrence of delayed degradation and thus the possible existence of a link between pesticide dissipation and microbial growth. It must be kept in mind that the CDE and Monod simulations must be considered as a working hypothesis rather than a definitive answer. This came to expression in the estimation of the Monod kinetic parameters. The obtained values had no significant biological value and are therefore of little value as no information was present on the initial biomass concentration.

Consequently caution should be addressed to future users of the Monod model, when little information is present on the matrix studied.

The experimental metalaxyl, isoproturon, and bentazone BTCs were well described using the transport model based on the convection-dispersion equation with first-order degradation or with delayed degradation using the Monod kinetics. Freundlich adsorption parameters and degradation parameters were fitted to the observed BTCs. The sorption strength of the different pesticides increased with decreasing mobility of the pesticides, which was in accordance with the results observed in batch degradation experiments. The sorption capacity of the substratums or the sorption strength of the pesticides was however lower in the column experiments. This difference in sorption between batch and column experiments could probably be attributed to a higher solid/liquid ratio in column studies, to an increase in surface area that could occur in batch from the disaggregation of clusters during shaking and moreover, to a continuously shaking of the pesticide-substratum solution which improves contact between pesticide and substratum[141]. This confirmed the idea that sorption coefficients obtained from batch experiments are often not suitable for describing solute transport at the column or field scale. They do however give an indication of the sorption capacity of a substratum.

The organic-carbon partition coefficient K_{oc} appeared to be a very good indicator of the mobility of the pesticide. Bentazone was the most mobile pesticide, followed by metalaxyl, isoproturon, and linuron. Metalaxyl and isoproturon, pesticides with similar mobility, leached more or less simultaneously, which indicates that pesticides with comparable mobility will behave in a similar way (degradation not taken into account). Further research should be performed with other pesticides to validate this hypothesis. If this hypothesis is valid, simulations could be performed to predict the leaching of a certain pesticide in the system, which could improve the efficiency of the system considerably. For example, water contaminated with pesticides with low K_{oc} values could be treated more carefully by reducing the flow on the system, thereby increasing the residence time and thus the opportunity for biodegradation. On the other hand, it could also provide guidelines for the construction of the biopurification system. If a farmer mainly uses mobile pesticides, an increase in the flow pathway (deeper phytobac, biobed or an extra container for a biofilter) will increase residence time.

An up scaling of the system from microcosm to macrocosm resulted in a similar or in some cases slightly lower retention of the four pesticides in the macrocosms compared to the microcosm system. The lower retention could be attributed to the lower substratum density, which decreases the amount of sorption sites.

A ranking in decreasing degradability of the pesticides in the micro- and macrocosms is as follows: linuron > metalaxyl-isoproturon > bentazone, which is similar to the mobility of the pesticides. Delayed degradation occurred for metalaxyl and linuron in the microcosms and for isoproturon, metalaxyl and bentazone in the macrocosms. The appearance of periods in which no or little degradation is observed, is typical in degradation kinetics of pesticides and has been designated as acclimation period or lag time. The appearance of a lag time is probably attributed to the proliferation of an initial small amount of pesticide degrading community to an amount which results in detectable pesticide degradation[136].

Degradation of metalaxyl and linuron in the macrocosms was confirmed in batch experiments, while isoproturon and bentazone did not show any degradation. The possibility exists that isoproturon and bentazone are partly retained as bound residue or degraded by white rot fungi. In addition, degradation of metalaxyl and linuron appeared to be more efficient in the upper part of the column, which was reflected in the lower half-live and shorter lag time for metalaxyl and linuron respectively. Furthermore, a lower degradation of linuron and metalaxyl was observed in batch experiments were non-treated organic matrix was used. This phenomenon known as accelerated degradation[134], could open perspectives to use the organic matrix of a biopurification system, which has been in use for some years, as an inoculation source for new systems to overcome slower degradation occurring during the start-up phase of the system.

Most mixes efficient in degrading or retaining pesticides were mixes containing dried cow manure. No significant differences could be found in retention and degradation of the mixes where no cow manure was added. The role of cow manure on pesticide degradation is not really elucidated yet and should be further elaborated. Moreover, decreasing the amount of soil, compared to the amount used in a classical biomix, did not reduce the efficiency of the system. A reduction in this mineral fraction would be advantageous as the cost of incinerating the material after use, increases with the soil fraction.

8.3.2 Does the inoculation of the biopurification system with pesticide primed soils enhances degradation of pesticides?

One of the observations from chapter 4 pointed out to the possibility to use organic matrix from a well established biopurification system as an inoculation source for a new system. This organic matrix appeared to have gained an increased ability to degrade linuron and metalaxyl in the batch degradation experiments. This phenomenon can be explained by the proliferation of an adapted community able to degrade the pesticide. The inoculation material does not necessary have to be organic matrix from a biopurification system, but can also be soil from a field previously treated with pesticides. Soils who developed the potential to degrade a pesticide, are also called pesticide-primed soils. The hypothesis in this chapter was that an inoculation with pesticide-primed material could enhance pesticide degradation in the system. To validate this hypothesis, column displacement experiments were performed in Chapter 5, where transport of metalaxyl and isoproturon was studied in the presence of metalaxyl and isoproturon primed material. The only variable in the organic matrix composition was soil, while the other substratums used were peat mix and straw at fixed ratios. These experiments were only performed on a microscale (Figure 8.1).

The results showed that the metalaxyl-primed material, originating from a biopurification system which had been in use for some years and was treated with metalaxyl, significantly increased degradation compared to a soil never treated with metalaxyl. Thus this material could serve as highly suitable material for the inoculation of new systems during the start-up phase. The start-up phase is the most vulnerable period for the biopurification system as higher leaching can occur when the original amount of biomass is too little to provoke sufficient degradation. Incorporating biomass which is already adapted, could reduce the duration of this start-up phase. The second advantage of the incorporation of material from a well established biopurification system, is a decrease in the amount of waste material after

use. This material is in some countries still considered as hazardous waste material which needs expensive treatments.

The amount of metalaxyl-primed material appeared to be of importance in the degradation of metalaxyl. A reduction from 5% to 2.5% reduced the degradation of metalaxyl. Thus a significant amount of biomass should probably be present to improve the degradation efficiency.

On the other hand, inoculation of the biopurification system with pesticide-primed material was not always successful. The use of isoproturon treated soil did not appear to enhance isoproturon degradation. The possibility exists that the soil lost its capability to degrade isoproturon as it was treated too long ago. The fact that metalaxyl is more easily degraded than isoproturon in the presence of pesticide primed soil, may also be related to the type of pesticide. Sorensen *et al.*[180] observed that mineralisation of recalcitrant pesticides such as the phenylureas, is restricted to a small group of organisms. This is in contrast to more easily degradable compounds such as MCPA that are metabolised by a broad range of soil microorganisms. Thus degradation of metalaxyl could be facilitated by a broad spectrum of microorganisms, in contrast to isoproturon, which needs more specific isoproturon degrading microorganisms.

Columns containing isoproturon-primed soil also appeared to develop the ability to degrade metalaxyl. This indicates that the metalaxyl degrading microorganisms in the isoproturon-primed soil were present at low concentrations or that microorganisms developed the ability to degrade metalaxyl. Thus adaptation might also occur. However, the use of this material increased the lag time in batch degradation experiments compared with metalaxyl-primed material.

These experiments provide an indication about the possible use of previously treated pesticide soil or biopurification matrix, as an inoculation source in the set-up of a new biopurification system. As this strategy was only successful for one of the two studied pesticide, additional research is necessary to gain insight in the applicability of this method and to further refine this strategy for practical use. Specific research points of interest are (i) the suitability of a mixture of pesticide-primed soils to treat a cocktail of pesticides coming onto a biopurification system, (ii) a more profound study on the minimal amount of pesticide-primed soil needed to enhance biodegradation[192], (iii) mutual interactions between populations, for example, the presence of dominant populations which could compete with smaller populations, (iv) the study of pesticide-primed soils to treat recalcitrant pesticides, since the possibility exists that these can only be degraded by a small spectrum of microorganisms.

8.3.3 How does a variation in flux influences the retention and degradation of pesticides in a biopurification system?

All previous column displacement experiments operated at the same flux. This flux is however very liable to several external factors. The flux consists of a hydraulic and chemical fraction. The hydraulic fraction may vary drastically according to the season, the type of spraying machine, the applicator, the crop, *etc*. The chemical fraction also depends on the

type of spraying machine, frequency of spraying, spraying scheme, behavior of the operator, *etc*. Both fractions may have a considerable influence on the efficiency of the system and will also to a large extent determine the type and/or dimension of the biopurification system.

To answer the question whether the flux influences the behavior of pesticides, column displacement experiments were performed in micro- and macrocosms with metamitron, bentazone, metalaxyl, isoproturon and linuron at three different fluxes in Chapter 6. In addition, to verify the observations found in Chapter 5, a mixture of five pesticide-primed soils was used in the organic matrix at all flows. The mixture consisted of soils previously treated with the studied pesticides. An additional set of columns containing a reference soil (not treated) was setup at the intermediate flow and treated with the same pesticides, to compare them in their degradation efficiency.

In microcosms, the sorption of fairly mobile pesticides (metamitron, isoproturon and metalaxyl) decreased considerably with increasing flux, due to nonequilibrium sorption at the highest flux. On the other hand, an increasing flux did not appear to have a significant influence on very mobile (bentazone) or immobile (linuron) pesticides, as respectively, breakthrough occurred very fast or did not occur in the time frame studied. Compared to the microcosms, very limited breakthrough occurred for the fairly mobile pesticides (metamitron, isoproturon and metamitron) and linuron in the macrocosms. The influence of flow on bentazone breakthrough was minimal and similar to the microcosms.

Degradation of the fairly mobile pesticides was also significantly influenced by the flux in the microcosms. Pesticides which are strongly retained (linuron) or which have a high leaching potential (bentazone) appear to be less submissive to the influence of water flux on degradation. The opportunity time, that may be required for maximum degradation, decreases as a result of faster transport of a pesticide through the organic matrix at a high flux. Moreover, a higher input of pesticides can lead to the presence of toxic levels to the pesticide degrading microbiota or a higher flow might lead to a distribution of the pesticide degrading biomass. Degradation parameters could not be determined for the majority of the pesticides in the macrocosms, except for bentazone and metalaxyl. Just as in the microcosms, a higher degradation could be observed with decreasing flux. The higher degradation observed in the macrocosms compared to the microcosms, may be attributed to a longer flow path, which increased the residence time and hence the opportunity for biodegradation. Moreover, the flux expressed in liters per day per volume of substratum ($L\ d^{-1}\ m^{-3}$) was higher in the microcosms, which results in a higher hydraulic and chemical load on the microcosms.

From the macrocosms, samples were taken at various points in time from the upper part of the matrix to perform batch degradation experiments. These experiments provide information on the pesticide degrading community dynamics by the determination of the lag time or acclimation period. No degradation of bentazone could be observed, which indicates that most of the bentazone dissipation observed in the columns will be caused by an increase in bound residues or by populations or species not able to grow under the conditions applied in batch degradation experiments. The lag time of metamitron and linuron decreased drastically in time for all flows, indicating a growth in the pesticide degrading community. This is in contrast to isoproturon and metalaxyl, where an increase in lag time could be observed in time for all flows. From the batch degradation, it could be

concluded that the influence of flow on the lag time was minimal, certainly after 123 days of pesticide application. Moreover, the inoculation of the pesticide-primed soil had a little surplus value on degradation compared to the reference soil. The inoculation of pesticide-primed soil could be beneficial in the removal of some pesticides during the start-up phase, as a lower lag time could be observed for metalaxyl and metamitron in the macrocosms with pesticide-primed soil at the start of the experiment. More or less the same accounts for the addition of reference soil in the microcosms. It did not enhance degradation of the studied pesticides significantly, with the exception of metalaxyl where a higher degradation in the presence of pesticide-primed soil could be observed.

For all flows studied, it could be stated that an increase in flow will be most detrimental for the treatment of very mobile pesticides, such as bentazone in micro- and macrocosms. In the microcosms, where the flow varied from 56.3 to 160.1 $L\ d^{-1}\ m^{-3}$, the increase in flux had a negative influence on retention and degradation of most pesticides (except linuron). While in the macrocosms, where the flow varied from 12.5 to *56.4* $L\ d^{-1}\ m^{-3}$, little breakthrough occurred for all pesticides, except bentazone. The flows applied in the macrocosms approached the average flow applied on a biofilter (20 $L\ d^{-1}\ m^{-3}$) most closely.

Further experiments could be carried out at lower flows, to profoundly reduce the presence of mobile pesticides in the effluent. A further decrease in the flow will however significantly decrease the capacity of the biopurification system.

A general remark for all column displacement experiments carried out, is that in these experiments, no labeled pesticides were used. Labeled pesticides, could provide more information on mineralization, the formation of metabolites and the formation of bound residues. These are processes which are not completely clear at this point.

8.3.4 How do pesticides behave in pilot biopurification system?

In the framework of the IWT 40727 project, which runs in collaboration with KULeuven and PCFruit, two pilot biopurification systems are constructed and will be followed up in 2009. These pilot installations are a continuation of the column displacement experiments, but on a larger scale. This set-up consists of 3 units and will treat isoproturon, linuron, bentazone, metalaxyl and metamitron contaminated water at a concentration of 10 $mg\ L^{-1}$ at a flow of 20 $L\ d^{-1}$. The composition of the two pilot installations are identical, with the difference that one system contains a mixture of five pesticide primed soils (cfr. Chapter 6) while the other one contains a non-treated or reference soil. This experiment will be performed to validate the previously found observations.

8.4 Matrix processing

8.4.1 How can pesticide residues in the organic matrix be reduced after use?

The observations in Chapter 4, 5 and 6 show that the majority of the pesticides are quite efficiently retained and degraded in the organic matrix from a biopurification system. Nonetheless, this matrix will mineralize slowly in time, thereby causing a depletion in nutrients, which leads to a reduction in efficiency of the biopurification system. At that moment, the organic matrix should be replaced.

What to do with the spent biopurification matrix? This matrix may partially be used to inoculate the new biopurification matrix, since it will already contain pesticide degrading communities. However, there will still be a rest fraction containing possibly high concentrations of pesticides. In Belgium, this rest fraction is still considered as hazardous waste and thus needs special treatments, which can be quite expensive.

One option is to incorporate the matrix in the field after use. However, some concern has risen about the possible presence of pesticide degrading microorganisms in this matrix. The apprehension is that the spread of the matrix in the field could lead to a proliferation of the pesticide degrading microorganisms resulting in a reduced efficacy of the applied pesticide on the field. Incorporation of the biopurification matrix is however up till now not yet regulated. Nonetheless, incorporation of 1 m^3 of biopurification matrix in the upper 30 cm of a 1 *ha* field, gives a dilution of 1:3000, which results in a very high spatial distribution of the community and thus in a considerable lower efficacy.

Composting, which is regarded as an effective and environmentally friendly strategy, could provide a solution for the treatment of this waste material. To test the ability of composting on the removal of retained pesticides in the organic matrix, small and large scale composting/incubation was carried out. Small scale incubation refers to composting in bins or barrels, while full scale composting is performed on an industrial scale in tunnels. The evolution in time of bentazone, linuron, metalaxyl and bifenthrin was followed-up in time.

In the industrial composting process a decrease in the concentration of metalaxyl, linuron and bentazone could be observed. Degradation of bifenthrin, the most persistent pesticide, was very limited. However, as the contaminated matrix was packed in a nylon bag and was thus not really blended with the composting feedstock (nevertheless, some exchange was possible via the gaseous and the liquid phase), the experiment should mainly be considered as an incubation test. Incorporation of the material into the compost heap could decrease the pesticide concentration more. This incorporation could however lead to contamination of the end product if complete degradation did not occur. In the small scale incubation process, a decrease in concentration was visible for bifenthrin, metalaxyl and bentazone. Dissipation could probably partly be attributed to the formation of bound residues. Additional studies with labelled pesticides, could give a decisive answer on that. The addition of dried cow manure did not appear to enhance the degradation of the pesticides present. As the contaminated matrix was already degraded to a large extent, composting of the

material was limited and therefore did not result in elevated temperatures normally occurring during a composting process. For that reason, a suggestion for future research could be the amendment of fresh material to initiate the composting process. Finally, it could be concluded that industrial and small scale composting/incuation did reduce the concentration of some pesticides during the time frame studied. However little removal was obtained for the persistent pesticide bifenthrin. Thus additional treatments and further study still appears to be necessary in order to discharge the material *e.g.* by incorporation in the field.

8.5 Additional remarks

The obtained results show that the biopurification system retains fairly mobile and immobile pesticides. The major problem lies in the treatment of very mobile pesticides such as bentazone. Their very low retention in the system, reduces the possibility for biodegradation. For these types of pesticides additional research is a necessity. If a removal of very mobile pesticides in the effluent is not possible with a biopurification system, the use of additional remediation techniques or the prevention of these pesticides entering the system should be considered.

Furthermore, the problem of the processing of the contaminated matrix still remains a major issue in the regularization of the system. Simple barrel composting without the addition of fresh substratums was not successful for all pesticides and thus needs additional attention.

Finally, these systems are studied and optimized for a standard agri- or horticultural farm which is characterized by a low hydraulic and a high chemical load. Diverse agricultural and horticultural sectors have however hydraulic and chemical loads which considerably deviate from an average farm by possible combinations of, on the one hand, a low or high chemical load and on the other hand a high hydraulic load. For these types of farms there is up till now no feasible solution for the treatment of pesticide contaminated water generated on-farm. Furthermore, specific application techniques and infrastructure creates a further differentiation. For example, after-harvest treatment plants generate high volumes of low contaminated water, while a contractor sprayer generates high volumes of highly contaminated water.

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Summary

The increasing use of pesticides in agriculture has given rise to a situation in which countries now have to cope with the problem of residues of these compounds in ground and surface water. 40-90% of surface water contamination is caused by direct losses (*e.g.* spills during filling operations, leakages of spray equipment, spray leftovers, *etc.*). Considering the cost of treating water to remove pesticides, contamination should be treated at the source, thus on-farm before discharging. On-farm biopurification systems, developed to treat pesticide contaminated water, consist of a biologically active matrix that retains pesticides into the organic matter and enhances their biodegradation. In order to optimize the efficiency of these systems, the fate of pesticides and the contribution of degradation and retention process needs to be well characterized.

In the present work, the intention was to unravel sorption and degradation processes on an increasing spatial scale. The first part of this thesis which focused on the sorption of pesticides with varying phyisico-chemical characteristics, was performed under static conditions. This means that transport of pesticides was not taken into account. For an optimal retention of pesticides, sorption of these compounds to the organic matrix should occur more or less instantaneously. Pesticides with a high organic carbon partitioning coefficient (K_{oc}) (*e.g.* linuron) showed a faster sorption, compared to the more mobile pesticides (*e.g.* bentazone). The organic matter content appeared to play a minor role in the sorption kinetics of the pesticides. Structural differences between the substratums were probably the reason for varying sorption kinetics of a pesticide. For example, sorption of pesticides to compost (fine) was in most cases faster than to coco chips (coarse). Simulations performed with HYDRUS-1D, showed that leaching of pesticides with a high mobility is more liable to nonequilibrium sorption. The second objective proposed in this optic, was to determine which organic substratums show the highest sorption capacity. Quantification of the sorption capacity with the Freundlich equation led to the following ranking with increasing sorption capacity: sandy loam soil < willow chopping, cow manure < straw, coco chips, compost < peat mix. However, in the selection of the substratums to be used in a biopurification system, the first criteria should be the function the substratum will fulfill in the system, followed by its sorption capacity. Finally, it could be observed that the sorption capacity was in this case strongly correlated with the organic carbon content, CaO content and the cation exchange capacity.

The second and major part of this thesis was performed under dynamic conditions, thus incorporating transport of pesticides. Experiments were performed in small or large scale columns. This experimental set-up aims at studying sorption and degradation processes at the same time. The first question posed in this regard, was how pesticides will behave in different matrix compositions. Sorption and degradation characteristics of linuron, bentazone, metalaxyl and isoproturon were quantified using inverse modeling techniques present in the transport model HYDRUS-1D. The sorption strength of the different pesticides

to the organic matrix, increased with decreasing mobility of the pesticides, which validated the results obtained under static conditions. The major difference between static and dynamic conditions was that a higher sorption capacity was observed in batch experiments. This led to the assumption that sorption coefficients obtained in batch experiments are not suitable for describing solute transport at the column or field scale. Concerning increasing degradability of the pesticides, pesticides could be ranked as follows linuron > metalaxyl-isoproturon > bentazone. Delayed degradation could be observed for some pesticides in the micro- and macrocosms. The time period with little or no degradation has been designated as acclimation period or lag time and could be fitted by implementing the Monod kinetics into HYDRUS-1D. Finally, concerning the composition of the mixture, it could be concluded that the addition of cow manure stimulated degradation of certain pesticides, and that a decrease in the soil fraction did not reduce the efficiency of the system.

Another observation from the previous column experiments indicated that organic matrix from a well established biopurification system could be used as inoculation source for a new biopurification system, as this matrix probably gained an increased ability to degrade the applied pesticides. To validate the hypothesis that an inoculation with pesticide-primed material enhances degradation of pesticides, column experiments were set-up where metalaxyl or isoproturon primed material was included in the matrix and transport of both pesticides was followed-up. This hypothesis was valid for degradation of metalaxyl in the presence of the metalaxyl-primed soil, but could not be verified in the case of isoproturon. This could be caused by a loss of capability to degrade isoproturon. On the other hand, the isoproturon-primed soil did develop the ability to decrease metalaxyl slightly. Thus, it was assumed that metalaxyl was degraded by a wide spectrum of microorganisms, while the degradation of isoproturon depends on the presence of specific communities.

The last part of the studies performed under dynamic conditions were carried out to gain some knowledge on the influence of a variable flux on pesticide degradation and retention. The flux, which consists of a hydraulic and chemical fraction, may vary in a biopurification system. To ensure proper working of the system when changes occur in the flux, column experiments were performed on a micro and macro scale at three fluxes to observe changes in degradation and retention. In microcosms, where a higher hydraulic and chemical load was applied compared to the macrocosms, sorption of intermediate mobile pesticides decreased considerably with increasing flux. This is in contrast to the macrocosms, where the three applied fluxes were lower and did not induce breakthrough of most of the applied pesticides. Degradation of the intermediate mobile pesticides was also significantly influenced by the flow in the microcosms. Due the limited amount of detectable effluent concentrations of the majority of the pesticides in the macrocosms, degradation parameters of these pesticides could not be determined. However, for the most mobile pesticide, it could be concluded that a higher flux influenced degradation negatively. Batch degradation experiments were performed with matrix samples from the macrocosms. These results indicated that the flux had only a limited influence on the acclimation period (lag time). An additional element in this study was the comparison between columns inoculated with a mixture of pesticide-primed soil and columns containing a non-treated soil. The incorporation of pesticide-primed soil appeared to be beneficial only for metalaxyl, where a slightly higher degradation could be observed.

Finally, we explored two strategies to reduce the amount of pesticide residues in the organic matrix after use. Contaminated matrix (bifenthrin, linuron, metalaxyl and bentazone) was treated in an industrial composting plant and in barrel incubation. A decrease in the non-persistent pesticides (linuron, metalaxyl and bentazone) could be observed in the industrial composting process. Removal of the persistent pesticide (bifenthrin) appeared to be limited. In the barrel incubation, a removal in concentration could be observed for the non-persistent pesticides metalaxyl and bentazone and also for bifenthrin. A removal in extractable pesticide concentration does however not always indicate degradation but could also be attributed to the formation of bound residues. Thus, it could be stated that the treatment of the matrix with the proposed composting process does not offer a decisive solution.

In conclusion, the results reported in this thesis have provided insight in the degradation and sorption processes occurring inside the biopurification matrix. These results can be used in future studies to further elaborate the behavior of other pesticides in biopurification systems and to formulate some preliminary guidelines for the use of a biopurification system. Examples of some preliminary guidelines are:

- The exclusion of very mobile pesticides in the biopurification system, or if they are applied, a drastic increase in flux should be maintained to provide sufficient time for biodegradation or sorption.
- Not to apply a very high flux as this is detrimental for the sorption and degradation of the majority of pesticides.
- The use of previously treated soils or matrix from a well established biopurification system as this could enhance degradation of certain pesticides.
- The use of organic substratums with a high sorption capacity (bearing in mind the function the substratum should fulfill in the system). For example, the use of willow chopping could increase leaching of pesticides, compared to the use of straw or coco chips.
- A decrease in hydraulic and chemical load during the start-up phase of the biopurification system is advised to provide time for the pesticide degrading community to proliferate.
- To add a small fraction of cow manure to the organic matrix, as this appears to stimulate degradation of certain pesticides.

Samenvatting

Het stijgende gebruik van pesticiden in de landbouw heeft geleid tot een situatie waarin verschillende landen te maken krijgen met pesticide residu's in het grond- en oppervlaktewater. 40-90% van de oppervlaktewaterverontreiniging wordt veroorzaakt door puntverliezen (bv. morsen tijdens het vullen van de spuittank, lekken van de spuittank, resterende spuitoplossing, enz.). Rekening houdend met de kosten die gepaard gaan met het zuiveren van met pesticiden vervuild water, zou deze vervuiling moeten aangepakt worden aan de bron. Op het bedrijf opgestelde biozuiveringssystemen, die ontwikkeld werden om met pesticiden vervuild water te behandelen, bestaan uit een organische matrix, die de pesticiden weerhoudt en hun biologische afbraak bevordert. Om de efficiëntie van deze systemen te optimaliseren, dient het gedrag van pesticiden in detail gekarakteriseerd te worden.

Het doel van deze thesis was om de sorptie- en degradatieprocessen van pesticiden in het biozuiveringssysteem te ontrafelen in systemen die toenemen in grootte. Het eerste deel van deze thesis is toegespitst op de sorptie van pesticiden met uiteenlopende fysico-chemische eigenschappen. Dit werd uitgevoerd in een statische omgeving. Hiermee wordt bedoeld dat er geen rekening gehouden wordt met het transport van pesticiden. Een optimale retentie van pesticiden aan het organisch materiaal dient min of meer onmiddellijk plaats te vinden. Een vertraging in het sorptieproces vergroot de kans op uitloging. In deze studie werd vastgesteld dat pesticiden met een hoge organische koolstof-verdelingscoëfficiënt (K_{oc}) (*e.g.* linuron) een snellere sorptie vertoonden, in vergelijking met meer mobiele pesticiden (*e.g.* bentazone). De hoeveelheid organisch materiaal bleek een zeer beperkte invloed te hebben op de sorptiekinetiek van de bestudeerde pesticiden. Structurele verschillen tussen de substraten lagen waarschijnlijk aan de basis van een variable sorptiekinetiek van een pesticide. Bijvoorbeeld, sorptie van pesticiden aan compost (relatief fijn materiaal) trad sneller op in vergelijking met sorptie aan coco chips (grof). Simulaties, uitgevoerd met HYDRUS-1D, toonden aan dat uitloging van pesticiden met een hoge mobiliteit meer onderhevig is aan niet-evenwichtssorptie. Na karakterisatie van de sorptiekinetiek, was er noodzaak om de sorptiecapaciteit van de verschillende substraten te beoordelen bij evenwicht. Een classificatie van de substraten met stijgende sorptiecapaciteit, op basis van de kwantificatie van de sorptiecapaciteit met de Freundlich isotherm, was als volgt: zandleem bodem < wilgenhaksel, koemest < stro, coco chips, compost < potgrond. Toch, tijdens de selectie van substraten die mogelijk kunnen gebruikt worden in een biozuiveringssysteem, is de functie die het substraat vervult in het systeem het voornaamste criterium gevolgd door de sorptiecapaciteit. Ten slotte, werd in deze studie vastgesteld dat de sorptiecapaciteit sterk gecorreleerd is met het organische koolstofgehalte, het CaO gehalte en de kationen uitwisselingscapaciteit.

Het tweede en voornaamste deel van deze thesis werd uitgevoerd in dynamische omstandigheden. Deze studies nemen het transport van pesticiden eveneens in

beschouwing. Experimenten in dit deel werden uitgevoerd in kolommen op kleine en grote schaal (micro- en macrokosmos). Met behulp van deze techniek, kunnen sorptie- en degradatieprocessen en hun interactie tegelijkertijd bestudeerd worden. De eerste studie in dit deel behandelt het transport van pesticiden in verschillende matrix-samenstellingen. Sorptie- en afbraakparameters van linuron, bentazon, metalaxyl en isoproturon werden gekwantificeerd via invers modelleren met het transport model HYDRUS-1D. De hoeveelheid pesticiden gesorbeerd aan de organische matrix, steeg met dalende mobiliteit van het pesticide. Dit bevestigt de resultaten waargenomen in de experimenten in statische condities. Het grote verschil tussen de resultaten bekomen in de statische en dynamische condities, was de grotere sorptiecapaciteit van de substraten in de statische omgeving. Dit leidde tot de veronderstelling dat sorptiecoëfficiënten bekomen in statische experimenten waarbij de pesticide-oplossing met het substraat continu geschud wordt, niet geschikt zijn om het transport van pesticiden in de kolom of in het veld te beschrijven. Op basis van de afbraakconstante en de halfwaardetijd, kunnen de pesticiden gerangschikt worden volgens dalende afbreekbaarheid: linuron > metalaxyl-isoproturon > bentazon. Vertraagde pesticide-afbraak kon vastgesteld worden voor sommige pesticiden in de micro- en macrokosmos. De tijdsperiode waarin geen of weinig afbraak optreedt, wordt ook wel aanpassingsperiode of lagtijd genoemd. Het verloop van pesticiden met een vertraagde afbraak, kan gefit worden met behulp van de Monod kinetiek die geïmplementeerd werd in HYDRUS-1D. Ten slotte, dient vermeld te worden dat de toevoeging van droge koemest de afbraak van bepaalde pesticiden blijkt te stimuleren en dat een afname in de bodemfractie de efficiëntie van het systeem niet reduceerde.

In de hierboven beschreven kolomexperimenten, werd eveneens vastgesteld dat de organische matrix uit een in gebruik genomen biozuiveringssysteem kan gebruikt worden als een inoculatiebron voor een nieuw biozuiveringssysteem. Deze matrix heeft waarschijnlijk de mogelijkheid verworven om de toegepaste pesticiden af te breken. Om deze hypothese te valideren, werden kolomexperimenten opgesteld waar materiaal (bodem of organisch materiaal), dat voordien behandeld werd met metalaxyl en isoproturon, toegevoegd werd aan de organische matrix. Transport van metalaxyl en isoproturon in deze bodems werd opgevolgd. De vooropgestelde hypothese kon bevestigd worden voor metalaxyl. Metalaxyl werd versneld afgebroken in aanwezigheid van het metalaxyl behandeld materiaal. Dit was niet het geval voor isoproturon, wat kan veroorzaakt worden door een verlies aan isoproturon afbraakcapaciteit van de isoproturon behandelde bodem. Anderzijds werd wel vastgesteld dat de isoproturon behandelde bodem de capaciteit om metalaxyl af te breken verworven had. Er werd verondersteld dat metalaxyl afgebroken wordt door een breed spectrum van micro-organismen, terwijl afbraak van isoproturon afhankelijk is van de aanwezigheid van specifieke populaties.

In het laatste deel van de studies uitgevoerd onder dynamische condities werd de invloed van een variabele belasting op de pesticideafbraak en -retentie nagegaan. De belasting kan worden opgedeeld in een hydraulische en chemische fractie. Deze fracties die terechtkomen op het biozuiveringssysteem zijn echter niet altijd constant. Om een goede werking van het biozuiveringssysteem te kunnen verzekeren wanneer de belasting op het systeem wijzigt, werden kolomexperimenten uitgevoerd op micro- en macroschaal. Hierbij werden drie verschillende belastingen gehanteerd om wijzigingen in retentie en degradatie op te volgen. In de microkosmos, waar een hogere hydraulische en chemische belasting werd toegepast in

vergelijking met de macrokosmos, werd een significant lagere sorptie vastgesteld bij een hoger debiet. Dit in tegenstelling tot de macrokosmos, waar de lagere hydraulische en chemische belasting geen doorbraak van de meerderheid van de pesticiden veroorzaakte. Afbraak van de relatief mobiele pesticiden in de microkosmos was lager wanneer een hogere belasting werd toegepast. Door de beperkte detecteerbaarheid van de meerderheid van de toegepaste pesticiden in het effluent van de macrokosmos, konden de afbraakparameters van deze pesticiden niet bepaald worden. Voor het meest mobiele pesticide (bentazon) kon echter wel vastgesteld worden dat een hogere flux de afbraak negatief beïnvloedt. Bijkomende afbraakexperimenten werden uitgevoerd met stalen van de organische matrix van de macrokosmos om een beeld te krijgen van de pesticide degraderende afbraakpopulaties via het bepalen van de lagtijd. De bekomen resultaten toonden aan dat de belasting slechts een beperkte invloed had op de lagtijd. Naast het bestuderen van de flux, werd eveneens voor één welbepaalde belasting een vergelijking gemaakt tussen kolommen geïnoculeerd met een mengsel van pesticide afbrekende bodems (bodems die afzonderlijk behandeld werden met de bestudeerde pesticiden) en kolommen waarin een niet-behandelde bodem werd toegevoegd. Inoculatie met de pesticide behandelde bodems bleek enkel een positief effect te hebben op de afbraak van metalaxyl.

Ten slotte werden twee strategieën onderzocht om de pesticidenresidu's in de organische matrix na uitgebruikname te reduceren. De matrix gecontamineerd met bifenthrin, linuron, metalaxyl en bentazon werd behandeld in een industriële composteringsfaciliteit en in ton compostering/incubatie. Een daling in de hoeveelheid niet-persistente pesticiden (linuron, metalaxyl en bentazon) werd waargenomen in het industriële composteringsproces. Reductie van het persistente pesticide, bifenthrin, werd niet waargenomen. Een reductie in de concentratie metalaxyl, bentazon en bifenthrin werd waargenomen in de ton incubatie. Een reductie in extraheerbare concentratie wijst echter niet altijd op afbraak, maar kan ook veroorzaakt worden door de incorporatie van pesticiden in de organische matrix. Er kon dus vastgesteld worden dat de behandeling van de matrix via de voorgestelde composteringprocessen geen sluitende oplossing biedt.

Als conclusie kan gesteld worden dat de bekomen resultaten een diepgaand inzicht bieden op de afbraak- en sorptieprocessen die plaatsvinden in een biozuiveringssysteem. Deze resultaten kunnen gebruikt worden in studies die het gedrag van andere pesticiden in het biozuiveringssysteem willen beschrijven en om een aantal preliminaire richtlijnen voor het gebruik van een biozuiveringssysteem te formuleren:

- De behandeling van zeer mobiele pesticiden in het biozuiveringssysteem moet vermeden worden. Indien ze toch toegepast worden, dient het debiet op het biozuiveringssysteem drastisch verlaagd te worden om genoeg tijd te voorzien voor microbiële afbraak.
- Het gebruik van een hoog debiet op het biozuiveringssysteem moet voorkomen worden aangezien dit nefast is voor de sorptie en afbraak van pesticiden.
- De inoculatie van een nieuw biozuiveringssysteem met een met pesticiden behandelde bodem of met een matrix van een biozuiveringssysteem in gebruik kan de afbraak van bepaalde pesticiden bevorderen.

- Gebruik maken van organische substraten met een hoge sorptiecapaciteit (rekening houdend met de functie die ze vervullen in het biozuiveringssysteem). Bv. incorporatie van wilgenhaksel kan de uitloging van pesticiden bevorderen in vergelijking met het gebruik van stro of cocochips.
- Een verminderde chemische en hydraulische belasting tijdens de opstartfase van een nieuw biozuiveringssysteem is aangewezen om meer tijd te bieden aan de pesticide-afbrekende populatie om zich te vermenigvuldigen.
- Het toevoegen van een kleine fractie koemest aan de organische matrix, kan de afbraak van sommige pesticiden stimuleren.

Curriculum Vitae

1. PERSONAL DATA

Name	Tineke De Wilde
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2. EDUCATION

1998-2003	Bio-Engineer, option agriculture (crop protection), Faculty of Bioscience Engineering, Ghent University, Gent
1992-1998	Secondary school, Science - Mathematics, Sint-Lodewijkscollege, Lokeren

3. PROFESSIONAL CARREER

2004-2005	Project Researcher (FOD Volksgezondheid, Veiligheid van de voedselketen en leefmilieu) at the Department of Food Safety and Food Quality – Laboratory of Food Chemistry (Prof. dr. ir B. De Meulenaer). Subject: 'Influence of conservation on different potato variaties on acrylamide formation during frying.'
2005-2009	PhD Researcher (IWT landbouwproject) at the Department of Crop Protection – Laboratory of Fytofarmacie) (Prof. dr. ir. W. Steurbaut). Subject: 'Biopurification of pesticide contaminated water generated on-farm.

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12 Jan - 19 Feb 2008	University of California, Riverside, CA, USA. Inverse modelling of pesticide breakthrough curves under the supervision of Prof. Jirka Simunek for the PhD thesis.
26 Aug - 29 Sep 2002	Institute of Agricultural Research for Development (IRAD), Buea, South West Province, Cameroon. Field research and collection of plant material and pathogens for the M. Sc. thesis.
12 Feb - 31 Jun 2002	Universidad Politécnica de Valencia, Valencia, Spain. Erasmus student exchange program.

5. EDUCATIONAL ACTIVITIES

2004 - 2008	Guidance of the M.Sc. thesis of Grzegorz Michalski (2005), Wael Moussa (2005-2006), Elena Carretero Fernandez (2006-2007), Dimitri De Maerschalk (2007-2008), Kyawt Kay Khaing Tun (2007-2008), Angel Andrety Colmenarez Pernia (2008-2009).

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7. PRESENTATIONS AND POSTERS

Chemical Reactions in Foods V. New knowledge on chemical reactions during the processing and storage of foods. September 29th – October 1st, 2004, Prague, Czech Republic. (oral contribution)

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International Conference on fruit, vegetable and potato processing. November, 6th - 8th, 2005, Brugge, Belgium. (Oral contribution).

6th Simposio Latino Americano de Ciëncia de Alimentos. November, 10th – 14th, 2005, Campinas, São Paulo, Brasil (Poster).

58th International Symposium on Crop Protection. May, 23, 2006 Gent. (oral contribution).

11th IUPAC International Congress of Pesticide Chemistry. August 6th – 8th, 2006, Kobe, Japan (Poster).

COST action 629. Water pollution in natural porous media at different scales: fate, impact and indicators, 4-5/09/2006, Cagliari, Italy. (oral contribution).

4th European conference on pesticide and related organic micropollutants in the environment/ 10th symposium on chemistry and fate of modern pesticides. November 26th – 29th, 2006, Almeria, Spain (Oral contribution).

The ninth international in situ and on-site bioremediation symposium. May 7th – 20th , 2007, Baltimore, Maryland, USA (Oral contribution).

59th International symposium on crop protection. May 22th, 2007, Ghent, Belgium (Poster).

2nd European biobed workshop, December 11th – 12th, 2007, Ghent, Belgium (Oral contribution).

2nd HYDRUS workshop, March 28th, 2008, Prague, Czech Republic (Oral contribution).

SETAC Europe 18th Annual meeting, May 25th-29th, 2008, Warsaw, Poland (Oral contribution).